NATIONAL INSTITUTE OF DIABETES & DIGESTIVE & KIDNEY DISEASES

NIDDK

Recent Advances & Emerging Opportunities

FEBRUARY 2005

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
National Institutes of Health
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CONTENTS

Message from the Director

The National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) conducts and supports research to uncover the biological bases for health and disease, and to promote rapid translation of this knowledge into clinical interventions to treat, prevent, or cure diseases within the Institute's mission. In this year's booklet, our fifth annual compendium of NIDDK-supported basic and clinical highlights of research advances and opportunities, we have chosen to underscore the translational aspect of our research portfolio. In describing the translation of insights gained at the laboratory bench into improvements in clinical care at the patient's bedside, we reaffirm that the ultimate goals of biomedical research supported by the Institute include not only the accumulation of knowledge, but also its application to improve the lives of patients who suffer from debilitating and costly diseases.

In December 2003, I established a Translational Research Working Group at the NIDDK, and charged it with identifying obstacles to translational research, developing a process to prioritize translational initiatives, identifying areas where resources would have broad application, and developing ways to address any obstacles to moving forward. As an outgrowth of this process, the NIDDK has now developed several initiatives designed to promote translational research into disease-specific as well as trans-Institute areas. One planned initiative will spur research into identifying new biomarkers—small molecules that are indicative of a disease and readily-measured—for well-defined human diseases for which few, if any, markers exist. For example, biomarkers for fibrosis would be of benefit in a number of diseases, including liver and kidney disease. They would facilitate and make more efficient the conduct of clinical trials and offer hope of earlier intervention in diseases before irreparable organ damage has occurred. Another initiative will seek to apply recent advances in basic research on protein synthesis and processing to a number of diseases caused by defects in the molecular folding of proteins, such as cystic fibrosis. Similarly, the development and validation of new animal models would provide useful tools for determining ways to apply fundamental scientific discoveries to NIDDK-relevant diseases. Yet another effort would support the development of therapeutic approaches to prevent the accumulation of reactive oxygen species, which are induced by elevated blood sugar, as a way to prevent complications of diabetes. Together, these and other initiatives will significantly strengthen the NIDDK translation research portfolio.

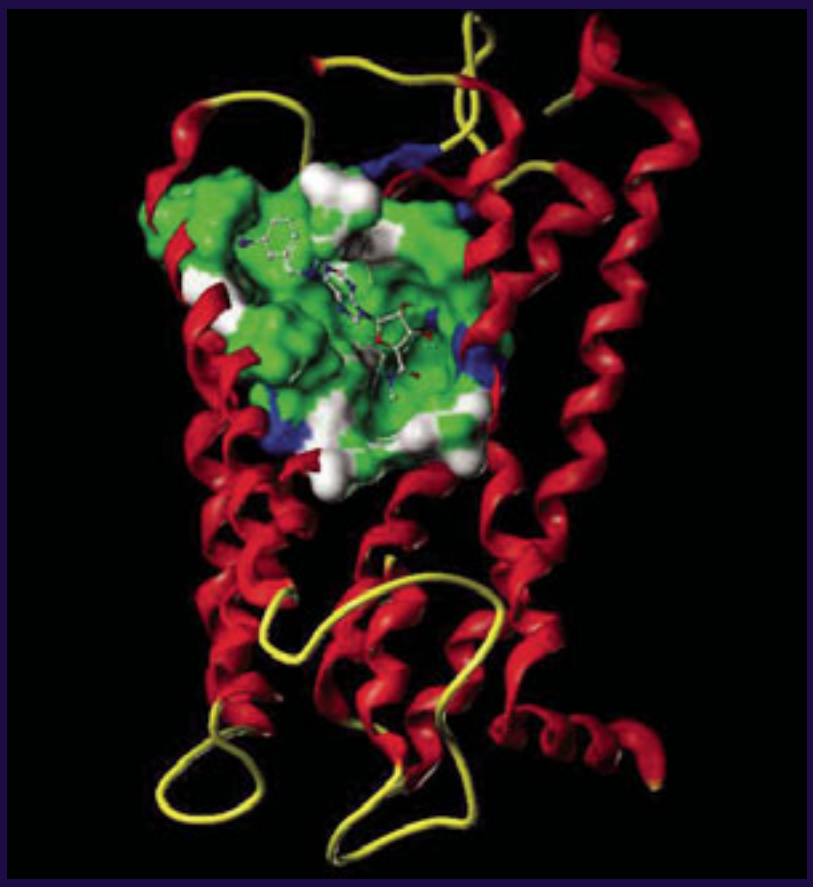
We are also working to capitalize on the research investments made possible by the doubling of the NIH budget between 1999 and 2003. With these resources, the NIDDK launched a range of consortia, networks, and clinical trials, while maintaining a strong portfolio of investigator-initiated research. In the post-doubling era, we must extract maximum benefit from this significant investment through the establishment of repositories for biological material collected as part of these studies. These repositories will facilitate additional future research by permitting the extensive use of samples and data collected from previous and future trials. The Institute is also encouraging ancillary studies to existing clinical trials as a way to derive an added benefit from well-characterized sets of patients and biological samples. Research consortia will continue to have a synergistic effect by bringing together scientists to address specific outstanding questions and by pooling scientific talent and resources. Moving forward, these types of efforts will become an increasingly important way for the Institute to make its previous investments the engine of future research opportunities.

The NIDDK has also taken an important role in several projects emerging from the NIH Roadmap for Medical Research. This effort, spearheaded by NIH Director Dr. Elias Zerhouni, is a framework of priorities designed to deepen our understanding of biology, stimulate interdisciplinary research teams, and reengineer clinical research to accelerate medical discovery and improve people's health. The NIDDK has taken a leadership position for the initiative on "Metabolomics Technology Development." The purpose of this initiative is to promote the development of highly innovative and sensitive tools for studying the "metabolome"—amino acids, peptides, lipids, and other small molecules. New technologies, propelled by this initiative, are expected to be broadly relevant to metabolic and other diseases within the NIDDK research mission. Another Roadmap initiative on "Interdisciplinary Research" aims to overcome the current barriers that prevent experts from different fields from working together to advance medical research. Obesity—which is a serious risk factor for type 2 diabetes—is a key example of a disease that could benefit from increased partnerships among different communities. The recent increase in obesity has been fueled by a complex interplay of environmental, social, economic, and behavioral factors, acting on a background of genetic susceptibility. This complex problem requires a multifaceted research effort.

In an effort to enhance communication with our research and patient community, this past year the NIDDK launched a new electronic newsletter, the "NIDDK Director's Update." Approximately twice a year, this update is sent to our National Advisory Council members and to constituency organizations, including patient advocacy groups, disease-specific organizations, and professional organizations. The Director's Update includes information about recent NIDDK activities, as well as on NIDDK-specific plans and trans-NIH issues of importance to the Institute. The NIDDK is also supportive of the NIH Public Trust Initiative, one aspect of which is to promote public participation in NIH-supported clinical research through enhanced transparency.

The research highlights that follow provide a snapshot of the important ongoing work being carried out by an immense network of basic scientists, clinical researchers, and patient volunteers. It is our hope that these advances provide an exciting and promising reflection of the NIDDK's many contributions to the national biomedical research enterprise.

Allen M. Spiegel, MD
Director, National Institute of Diabetes and Digestive and Kidney Diseases
National Institutes of Health
Department of Health and Human Services



In research that may impact the treatment of several diseases, scientists are using molecules that can bind to and activate (or inhibit) human proteins called adenosine receptors to study receptor signaling. Shown is a computer-generated model illustrating how one such activating molecule (an “agonist”) binds to one subtype of adenosine receptor. The agonist is represented as a very small ball-and-stick model within its binding site on the receptor. For more information on this exciting research, see the Feature on “Insights into Cellular Communication Through Medicinal Chemistry.” Image courtesy of Dr. Ken Jacobson, Chief, Molecular Recognition Section, NIDDK Division of Intramural Research.

Cross-Cutting Science

The tale of scientific discovery is told in incremental steps, with each new insight adding another page to the story. Often, this research focuses on the fundamental, microscopic and molecular components of an organism—its DNA, genes, proteins, and metabolites—and the exquisitely complex ways in which these elements are organized, regulated, and interact. While its ultimate application may not always be immediately obvious, this research can form a crucial foundation for future investigations, and insights gained from this research can be expected to facilitate disease-based research in a wide range of fields. A critically important aspect of cross-cutting research is the effort to translate research advances made in the laboratory into more effective therapies for patients, and to use insights gained from clinical studies to spur novel research directions in the laboratory.

STRENGTHENING RESEARCH ACROSS THE NIDDK

From Bench to Bedside – New Efforts to Accelerate Translational Research: Shepherding research from the laboratory—the "bench" at which a scientist performs experiments—to the doctor's office or hospital room—the "bedside" at which the patient is treated—is an important component of trans-NIDDK research. "Bench to bedside" is one aspect of "translational research," the movement of knowledge gained from laboratory research studies into the realm of clinical studies. Translation can also refer to efforts to bring insights gained from clinical trials to changes in the practice of medicine on a large scale to effect improvements in public health. Ultimately, investigators seek to move research findings from clinical study to healthcare practice in the community or public-health arena, a progression termed "bedside to practice."

The reverse process, "bedside to bench research," (or bench to bedside and back) is also valuable, as it brings knowledge gained in a clinical setting back to the laboratory for further exploration that may in turn spur new clinical endeavors. Ideally, knowledge flows in both directions, with research insights translating into improvements in patient care, and with clinical studies and observation catalyzing new lines of investigation at the laboratory bench. Translational research is an overarching theme of this document, highlighted especially in the Stories of Discovery and other advances in this document, and represented on the cover.

The NIDDK has undertaken new efforts to bolster translational research. In early FY 2004, the NIDDK Director established the Translational Research Working Group to identify obstacles to translational research and to develop ways of overcoming them, to identify opportunities, and to assess translational research priorities. Although the Working Group is focused on bench-to-bedside research, the NIDDK also vigorously supports translational research from bedside to practice. Such efforts include, for example, ongoing research demonstration and dissemination projects to explore strategies to effectively bring results of major clinical trials to patient care and the public, and an FY 2004 conference to discuss the science of translating diabetes and obesity research from clinical trials to the community and future research directions.

In developing new translational research efforts, the Translational Research Working Group sought external input from the NIDDK's National Advisory Council through discussions at its 2004 meetings, and other sources. Key areas for intensified research that emerged include:

- Enhancing the development of biomarkers (for example, particular biological molecules or patterns

that would reflect disease progression prior to the appearance of actual disease symptoms and that could thus improve monitoring of the effects of experimental treatment strategies);

- Developing new imaging methods;
- Generating new animal models for preclinical research on NIDDK-relevant diseases;
- Research on angiogenesis (blood vessel growth) for diabetic complications and islet transplantation;
- Research on an important effect of elevated blood sugar levels—the overproduction of reactive oxygen species in cellular components called mitochondria;
- Developing therapeutic agents for diseases characterized by protein misprocessing and misfolding; and
- Further research on a type of molecule called RNAi, which may have therapeutic potential.

Many of these areas would have implications for diseases across the mission of the NIDDK.

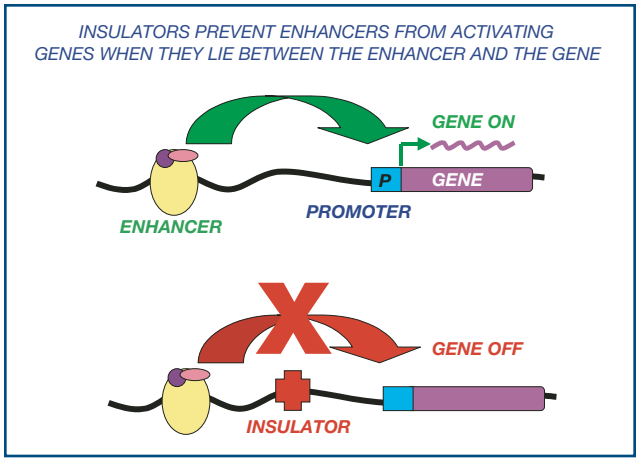
New Cross-cutting Efforts: The NIDDK is pursuing new broad-based opportunities to advance research on many diseases. For example, an initiative launched this past year is encouraging research to understand and mitigate issues of health disparities in diseases within the mission of the NIDDK. Another new initiative will support projects that advance the use of "proteomic" approaches in research on diabetes, obesity, and endocrine, digestive, kidney, urologic, and hematologic diseases. Proteomic technologies focus on proteins, just as genomic approaches focus on genes (and specifically on the DNA of which genes are made, and on the RNA intermediates between genes and the proteins they ultimately encode). Proteomics will help shed new light on biological processes in health and disease by enhancing understanding of patterns of protein production, interactions among proteins, and other aspects of proteins in cells, tissues, and organ systems. Also relevant to diseases across its mission, the Institute began an initiative in 2004 to encourage the development of assays for high throughput drug screening.

As part of the NIDDK's Division of Intramural Research, three laboratories—devoted to chemical synthesis, biological screening, and computational chemistry—are working together on a "Chemical Biology" initiative to develop small molecule reagents. These reagents would first be used in NIDDK scientists' research, and the reagents could eventually be optimized further for potential use as drugs. This initiative fosters interdisciplinary research approaches to important biological problems.

Research Training – Ensuring a Pipeline of Investigators for the Future: A critical component of NIDDK's research efforts is investigator training. Among the Institute's research training efforts are those focused on minority investigators, to advance scientific knowledge and to help reduce racial and ethnic health disparities. The Institute's Office of Minority Health Research Coordination established the Network of Minority Research Investigators (NMRI). The major objective of the NMRI is to encourage and facilitate participation of members of underrepresented racial and ethnic minority groups in the conduct of biomedical research in fields relevant to the NIDDK's mission. The NMRI is a communication network of current and potential biomedical research investigators and technical personnel from traditionally underserved communities: African American, Hispanic American, American Indian, Alaska Native, Native Hawaiian, and other Pacific Islanders. The major objective of the network is to encourage and facilitate participation of members of underrepresented racial and ethnic minority groups in the conduct of biomedical research in the fields of diabetes, endocrinology, metabolism, digestive diseases, nutrition, kidney, urologic, and hematologic diseases. A second objective is to encourage and enhance the potential of the underrepresented minority investigators in choosing a biomedical research career in these fields. An important component of this Network is promotion of two-way communications between network members and the NIDDK. The primary goals of the Network are to help minority investigators achieve career success while working on issues concerning health-related racial and ethnic disparities.

The NIDDK also supports educational efforts at the college and pre-college levels. For example, the Diabetes-based Science Education in Tribal Schools (DETS) Program is intended to promote a diabetes-based science curriculum that will enhance understanding and appreciation of the problems of diabetes in American Indian communities, and will stimulate general student interest in diabetes-based science in the early years (pre-college) of education. Selected tribal colleges and universities have been funded to develop supplemental curricula for K-12 schools in American Indian and Alaska Native communities. The investigators have embarked on developing three parallel curricula, K-4, 5-8 and 9-12, which will be sequential and interrelated to give a continuum of exposure to diabetes-based science education. The NIDDK, the Centers for Disease Control and Prevention (CDC), and the Indian Health Service (IHS) are jointly supporting the program. Through better understanding of diabetes, tribal children can be instrumental in preventing the development of and in better managing diabetes, and reducing its human costs. This goal can be better achieved through the entry of greater numbers of tribal children into the health-science professions.

Among other training programs is the NIDDK's Summer Internship Program (SIP), which provides an opportunity for undergraduate students to participate in research under the direction of preceptors in NIDDK laboratories. The purpose of this program is to advance the state of biomedical knowledge, and to introduce the students to current laboratory methods in the field. The National High School Student Summer Research Program is designed to enhance exposure to basic and clinical research, and academic medicine, as viable and desirable career choices, among a pool of underrepresented minority high school students. Such a program serves as an early component of the pipeline that brings students from economically disadvantaged backgrounds and medically under-served populations to the corridors of academia, which, in turn, focus on producing scientists, physicians, and allied health professionals to practice in their respective communities.



"Insulator" elements are naturally-occurring segments of DNA that mark boundaries in certain regions of the genome so as to enable a cell to regulate neighboring genes independently. Insulator elements have two functions, the first of which is referred to as enhancer-blocking activity, as shown. An enhancer is a sequence of DNA that directs the cell to turn on an associated gene. If positioned near an enhancer, an insulator can prevent the enhancer's signal from being broadcast in the wrong direction and thus keep the cell from turning on genes that it shouldn't. The second function of insulators is to set up a "barrier" to prevent unwanted silencing—or tight shutting off—of genes. Image courtesy of Dr. Gary Felsenfeld, NIDDK Division of Intramural Research. **For more information, see page 9.**

NIH Roadmap for Medical Research in the 21st Century: The NIH Roadmap provides a framework of the priorities the NIH as a whole must address in order to optimize its entire research portfolio. It identifies the most compelling opportunities in three main areas: New Pathways to Discovery, Research Teams of the Future, and Re-engineering the Clinical Research Enterprise. The following are highlights of a few of the many Roadmap initiatives.

The NIDDK has a leadership role for an NIH Roadmap initiative on "Metabolomics Technology Development." The "metabolome" is the complete set of small molecules in the body which function as nutrients, chemical signals and building blocks such as amino acids, peptides, and lipids. "Metabolomics" is the study of these small molecules. The purpose of this initiative is to promote the development of highly innovative and sensitive tools for studying metabolomics. The development of such novel

technologies can directly benefit the study of diseases within the NIDDK mission. For example, metabolomics could lead to the identification and validation of surrogate markers that correlate with stage or rate of progression of diabetes and its complications. Furthermore, metabolomics technologies could be applied to the development of novel, less-burdensome diagnostic tests for pre-diabetes and type 2 diabetes.

Another Roadmap initiative on "Interdisciplinary Research" aims to overcome the current barriers that prevent experts from different fields from working together to advance medical research. Obesity—which is a serious risk factor for type 2 diabetes—is a key example of a disease that could benefit from increased partnerships among different communities. The increase in obesity has been fueled by a complex interplay of environmental, social, economic, and behavioral factors, acting on a background of genetic susceptibility. Therefore, researchers with expertise in numerous disciplines—such as genetics, behavioral science, and biochemistry—can offer important contributions to obesity research. Another example of an initiative that will benefit NIDDK programs is the establishment of "translational research core services" to promote translation of novel therapeutics from the bench-to-the-bedside by providing access to sophisticated manufacturing capacity and expert advice to ensure that drug-development regulations are observed.

BASIC RESEARCH – CLINICAL IMPLICATIONS

2004 Nobel Prize in Chemistry Awarded for the Discovery of Ubiquitin-Mediated Protein Degradation: The 2004 Nobel Prize in Chemistry was awarded to three scientists for their discovery of ubiquitin-mediated protein degradation, a process that regulates protein destruction inside a cell. The winners are Dr. Aaron Ciechanover and Dr. Avram Hershko of Technion-Israel Institute of Technology in Haifa, Israel; and Dr. Irwin Rose of the University of California, Irvine. Drs. Rose and Hershko have previously received funding from the NIDDK.

In the early 1970s, Dr. Irwin Rose, who was then at the Fox Chase Cancer Center in Philadelphia, and Dr. Avram Hershko of Technion, were independently studying various research areas related to protein activity in the cell and the relationship to various syndromes, disorders, and diseases. When the two men met at a scientific conference at the NIH in 1976, they realized that they were both interested in the same research question: How does the cell select certain proteins for disassembly into their component parts, contributing to the regulation of protein activity within the cell? Much of the research in the field of cellular biochemistry up to that point had focused on the production of proteins in the cell, not the destruction of them. What interested both men was why it took energy (in the form of adenosine triphosphate, ATP, the energy currency of the cell) to break down these proteins, when in every other arena of biochemistry (such as digestion) energy is not needed for protein degradation. This enigma was too irresistible for the two scientists to ignore.

Out of this interest grew a research collaboration between Drs. Hershko and Rose, and later Aaron Ciechanover, one of Hershko's graduate students at Technion. The trio worked on the problem separately and together during several summer sabbaticals that Drs. Hershko and Ciechanover spent as visiting scientists in Dr. Rose's laboratory at Fox Chase Cancer Center.

What they discovered was that protein degradation in the cell is a highly selective activity regulated through a multi-step process that involves "tagging" a protein slated for destruction with a marker. Once a protein undergoes this process, the tagged protein is then chaperoned to the proteasome, the garbage disposal of the cell, for disassembly into its amino acid parts, which can then be reused to make other proteins. Drs. Ciechanover, Hershko, and Rose discovered that the marker that tagged proteins for destruction was the protein ubiquitin, a small polypeptide of only 76 amino acids.

Ubiquitin was first isolated in 1975 from bovine thymus in an NIH-funded study and reported by Gideon Goldstein, *et al.*, in an article in which the authors referred to the newly discovered protein as "ubiquitous immunopoietic polypeptide (UBIP) because it is widespread and perhaps even universally represented in living cells" (*Proc Natl Acad Sci USA* 72: 11-15, 1975). It soon became apparent that this protein was a staple of eukaryotic cells (cells with a defined nucleus) and found in most higher organisms, including plants, fungi, yeast, and animals.

The researchers had hypothesized that the energy requirement for protein destruction was needed for the cell to maintain specificity and control over the process of protein destruction. The question was how the cell used the energy to do this. Drs. Ciechanover, Hershko, and Rose answered this question through a series of pioneering biochemical studies throughout the late 1970s and early 1980s that led to the elucidation of the ubiquitin-mediated protein degradation system. They made their seminal discoveries in 1979 and reported their findings in two papers published in 1980 in the *Proceeding of the National Academies of Science of the United States of America.*[1,2]

They discovered that the tagging of a protein for destruction was a multi-step process that begins with the creation of a stable, high-energy covalent bond between ubiquitin and an enzyme, labeled E1. The creation of this covalent bond, the strongest kind of chemical bond, requires the input of significant energy in the form of ATP. This was the energy requirement that had so puzzled Drs. Hershko and Rose at the outset of their research. The discovery of the role of covalent bonding surprised the researchers, because most protein-protein interactions that take place inside a cell involve temporary bonding between molecules through weak chemical attractions, such as hydrogen bonding.

The researchers showed that the next step in the process involves the transfer of the ubiquitin protein from the first enzyme, E1, to a second enzyme, E2. The E2-ubiquitin complex then binds to another complex—a third enzyme, E3, and its corresponding target protein, the protein slated for destruction. An important discovery was that it is the specificity of the E3 enzyme that determines which proteins are degraded. Although the E1 and E2 enzymes are relatively similar through all cell types and organisms, there are hundreds of different E3 enzymes within each cell, each one programmed to recognize and bind to a specific protein.

For a brief time, all four components are bound together in very close proximity. Then the ubiquitin binds to the target protein, releasing the E2 enzyme, followed by the release of the E3 enzyme. Through this process, the target protein is tagged with the ubiquitin molecule. Another surprise came when the researchers discovered that the process didn't stop there. The binding of one ubiquitin molecule to the target protein apparently was not enough. They discovered that the last step of the process is repeated many times until a long chain of ubiquitin molecules is attached to the target protein. They termed this part of the process "polyubiquitination." This ubiquitin chain chaperones the target protein to the proteasome where it acts as the passkey for entry. The proteasome assists with the entry of the target protein into the barrel-shaped organelle, meanwhile releasing the ubiquitin to go about its business. Once inside the proteasome, the target protein is degraded into short peptide chains of seven to nine amino acids.

Drs. Ciechanover, Hershko, and Rose had worked out most of the details of the multi-step ubiquitin-tagging process by 1983. However, other work later brought to light insights into the ways that ubiquitin-mediated protein degradation contributes to the

general homeostasis of the cell, as well as other roles of the ubiquitin protein in the cell. For example, not only does the cell use the ubiquitin system to modulate protein concentrations according to the need for protein activity, but recent research has shown the system's other roles. Up to 30 percent of newly-produced proteins are immediately destroyed using the ubiquitin system, possibly because they are poorly formed or malfunctioning. Ubiquitin is also an important player in the regulation of the cell cycle, DNA repair, maintenance of chromosome structure, programmed cell death (apoptosis), and immune and inflammatory reactions. Current and future research will focus on developing drugs to intercede at various points in the pathway, either to destroy unwanted proteins or to prevent the destruction of critically needed proteins.

The NIDDK supports a broad program of research on intracellular protein-protein interactions, including study of the role of ubiquitin in protein degradation and other cellular functions. Dr. Avram Hershko received funding from NIDDK, from 1980 to 1995, for the study of the mechanisms of intracellular protein breakdown. Dr. Irwin Rose received funding from the National Institute of Arthritis, Diabetes, Digestive, and Kidney Diseases, the predecessor of the NIDDK, from 1973 to 1982, for the study of carbohydrate metabolism and the control of hexokinase loss in diabetes.

[1] Ciechanover A, Heller H, Elias S, Haas AL, and A Hershko. ATP-dependent conjugation of reticulocyte proteins with the polypeptide required for protein degradation. *Proc Natl Acad Sci USA* 77: 1365-1368, 1980.

[2] Hershko A, Ciechanover A, Heller H, Haas AL, and IA Rose. Proposed role of ATP in protein breakdown: Conjugation of proteins with multiple chains of the polypeptide of ATP-dependent proteolysis. *Proc Natl Acad Sci USA* 77: 1783-1786, 1980.

2004 Albert Lasker Award for Basic Medical Research for Work on Nuclear Hormone Receptors: The 2004 Albert Lasker Award for Basic Medical Research was awarded to three scientists for the discovery of a superfamily of nuclear hormone receptors and elucidation of a unifying mechanism that regulates embryonic development and diverse medical pathways. The winners are Dr. Pierre Chambon of the Institute of Genetics and Molecular and Cellular Biology in Strasbourg, France; Dr. Ronald M. Evans of the Salk Institute for Biological Studies in San Diego, California; and Dr. Elwood V. Jensen of the University of Chicago and University of Cincinnati College of Medicine. Their award-winning research, funded in part by the NIDDK, was in the field of nuclear hormone receptors, an area of research of special interest to the Institute.

The winners received the award for the identification of a common cellular mechanism through which a diverse group of hormones, i.e., chemical signaling molecules, regulates a wide range of physiological responses throughout the life-span of an organism. Their research on rodents and humans showed how hormones regulate gene activity through the activation of the hormones' corresponding hormone receptors in the nucleus of a cell. The researchers discovered that there is a "superfamily" of hormones that have corresponding receptors in the nucleus of the cell. This superfamily includes steroid hormones, thyroid hormones, and fat-soluble molecules such as Vitamins A and D. There are also many known nuclear hormone receptors with unidentified corresponding hormone activators—the so-called "orphan nuclear receptors."

Dr. Jensen, who received significant funding from the NIH's National Cancer Institute (NCI), set the stage for this discovery, in the 1950s, with his work on estrogen, a steroid hormone. He discovered that estrogen can activate certain genes inside the nucleus of a cell by binding to its nuclear receptor. Later work by Dr. Jensen and others identified the nuclear receptors

for other steroid hormones, such as testosterone, progesterone, glucocorticoids, aldosterone, and Vitamin D, which has steroid properties. His research ultimately led him to investigate the role of estrogen receptors in breast cancer tumors and led to the use of tamoxifen, an anti-estrogen compound, for the treatment of breast cancer.

By the 1980s, Drs. Chambon and Evans had expanded on Dr. Jensen's work by examining how molecular endocrinology influences gene control—i.e., how hormones turn genes on and off. By early 1986, working independently, Drs. Chambon and Evans discovered the genes for two important nuclear hormone receptors, the estrogen and the glucocorticoid nuclear receptors, respectively. In the same year, they independently discovered a nuclear hormone receptor for retinoic acid, also known as Vitamin A. They later discovered that the receptor, which Dr. Evans named Retinoid X Receptor (RXR), had unique physiological properties and could be used to identify the corresponding hormone for many orphan nuclear receptors. Perhaps Dr. Evans' and Dr. Chambon's greatest contribution to the field of research was in the refining of this technique using RXR to identify orphan nuclear receptors' corresponding hormones. Many of the orphan nuclear receptor complexes that have been "adopted" through this technique have applications to research of specific chronic diseases, including type 2 diabetes and lipid-related disorders.

Many NIH components contributed funding to the research of Drs. Jensen, Evans, and Chambon, including the NIDDK, the NCI, the National Institute on Aging (NIA), the National Institute of Child Health and Human Development, the National Institute of General Medical Sciences, the National Heart, Lung, and Blood Institute, and the National Center for Research Resources. Dr. Ronald M. Evans is a Howard Hughes Medical Institute Investigator.

Nuclear Receptor Signaling Atlas (NURSA), A Trans-NIH Initiative

Currently, the NIDDK is leading an initiative to encourage research on nuclear hormone receptors and to apply discoveries in this field to other fields, including disease-targeted research. The Nuclear Receptor Signaling Atlas (NURSA) is a trans-NIH initiative designed to develop a comprehensive understanding of the structure, function, and role in disease of nuclear hormone receptors, with particular focus on metabolism and the development of a number of metabolic disorders, including type 2 diabetes, obesity, lipid dysregulation, and others, as well as in processes of aging and hormone-dependent cancers.

Dr. Evans leads NURSA as co-Director with colleague, Dr. Bert W. O'Malley of Baylor College of Medicine in Houston, Texas. The initial focus of NURSA was in the area of orphan nuclear receptors, but it soon expanded to include research on all nuclear receptors. The hope is that research into the role of nuclear receptors on genetic expression, and elucidation of the mechanisms of action through which this occurs, will lead to better understanding of some of the diseases that are at the core of the NIDDK and NIH mission—including obesity, diabetes and its complications, osteoporosis, hormone dependent cancers, and digestive diseases.

NURSA began as a request for applications (RFA) that was issued by the NIDDK in June 2001—"A Functional Atlas of Orphan Nuclear Receptors." The RFA was funded in August 2002 as a consortium agreement between the NIH and five institutions: Baylor College of Medicine, Salk Institute, Duke University, the University of Pennsylvania, and the University of Texas Southwestern. The NURSA consortium exists as a cooperative agreement comprising research projects and core resources, funded by three NIH institutes: the NIDDK, the

NCI, and the NIA. Today, NURSA comprises the three original NIH institutes and the five original academic institutions, as well as three other academic institutions: Van Andel Institute, the Beckman Research Institute, and the University of Rochester.

A Straightforward Chemical Modification with Profound Implications: What do toothpaste, salad dressing, and laundry detergent have in common with cutting-edge treatments for diseases such as Crohn's disease and hepatitis C? A lengthy molecule, polyethylene glycol. Used in food and household products as a thickener, polyethylene glycol—also known as PEG—is used by researchers and clinicians to improve the durability of drugs to treat a number of debilitating conditions, including several within the NIDDK research mission.

Chemically, PEG is a long chain of molecules containing carbon, hydrogen, and oxygen atoms. Because of the electric charges on these atoms, PEG attracts an extensive retinue of water molecules when dissolved in an aqueous solution, and the lengthy hydrocarbon chain is effectively "coated" with them. In the late 1970s, researchers found that attaching molecules of PEG to biomolecules—a process known as "pegylation"—increased the molecules' ability to dissolve in water and protected them from enzymatic degradation. In the body, pegylated molecules are protected from immune response and other clearance mechanisms, which has the effect of lengthening the time the drug persists in the body. This effect in turn means that people require fewer doses of a drug and that drugs can accumulate to higher therapeutic levels in the body than would be possible otherwise.

In 1987, NIDDK-supported scientists studying the rare, inherited disease adenosine deaminase (ADA) deficiency reported that weekly injections of pegylated adenosine deaminase could be an effective short-term treatment. Children with ADA deficiency are unable to properly metabolize the nucleotide adenosine because they lack a critical enzyme, and as a consequence, their immune systems break down. In extreme cases, a condition known as severe combined immunodeficiency can develop, and these patients may have to live in a protected environment to avoid exposure to infectious agents in the environment. At the time of the trial, the primary therapy for ADA deficiency was regular blood transfusions to provide the missing enzyme. However, these transfusions were not always effective, and they carried risks of iron overload or viral infection. The finding that ADA deficiency could be treated with once-a-week injections of pegylated adenosine deaminase was not only an important advance in the treatment of this serious disease, but also a demonstration of the viability of pegylation as a therapeutic strategy.

In the intervening years, pegylation has been used to prolong the beneficial activity of a wide range of drugs and has had a significant impact on the treatment of a number of diseases. For hepatitis C, a pegylated form of the protein interferon is now part of the standard therapy, along with an antiviral drug. Although many patients respond to this therapy, others (especially African Americans) have low response rates, and the virus continues to cause liver damage. The NIDDK is therefore sponsoring a clinical trial, HALT-C, to determine if long-term treatment with pegylated interferon therapy is beneficial to patients who have not responded to initial therapy. Additionally, industry is sponsoring a number of trials of pegylated agents, including a pegylated tumor necrosis factor-alpha antibody for the treatment of Crohn's disease.

Over the past 20 years, pegylation has evolved from a tool in the research laboratory to an effective approach to treatment augmentation. Pegylated drugs are generally safe and effective, and pegylation has emerged as the favored way to improve the staying power, and hence the effectiveness, of a variety of compounds. As for the future, studies are under way to examine the possible benefits of pegylating molecules such as insulin, to prolong its circulation time; antibodies for targeting of tumors; and other enzymes to aid in recovery from injury.

Marking Boundaries on the Blueprint of Life:
At any given time, in any given type of cell, some genes are kept off, while others are turned on, that is, actively read by the cell to generate the products they encode. A group of NIDDK's intramural scientists has been elucidating one way by which this differential regulation is achieved: the activity of "insulator" elements. Insulator elements are segments of DNA that mark boundaries in certain regions of the genome so that neighboring genes can be regulated independently. Originally discovered in fruit flies, insulators also exist in animals and humans. In their investigations, the scientists particularly focused on a pair of insulators that flank a set of beta-globin genes (beta-globin is used in blood cells).

Insulator elements have two functions, the first of which is referred to as enhancer-blocking activity. An enhancer is a sequence of DNA that directs the cell to turn on an associated gene. If positioned near an enhancer, an insulator can prevent the enhancer's signal from being broadcast in the wrong direction and thus keep the cell from turning on genes that it shouldn't. Through studies of an insulator near the beta-globin locus, the scientists discovered a core segment responsible for the enhancer-blocking activity. By attracting a protein called CTCF and other factors that work with it, this part of the insulator keeps an enhancer focused on the correct gene. In other experiments, the scientists had discovered that insulators and CTCF also play an important role in a regulatory phenomenon called "imprinting," which controls certain genes. An imprinted gene is turned on or off depending upon whether the chromosome on which it's located was inherited from the mother or father. The scientists examined a different chromosomal region (not the beta-globin locus) containing an imprinted gene with an insulator element between it and a nearby enhancer. Through a complex process, cells modify the insulator only on the paternally-inherited copy of this chromosome. This modification restricts access of CTCF to the insulator, and thus prevents it from blocking the enhancer.

The second function of insulators is to set up a "barrier" to prevent unwanted silencing—or tight shutting off—of genes. All of a cell's DNA, including the segment with beta-globin genes, is packaged to some extent with various proteins, called histones, into a structure called "chromatin." Chromatin serves not only to keep the cell's long strands of DNA from becoming tangled and unwieldy, but also as a dynamic structure that helps regulate whether and when genes are turned on or off. One way a cell can keep certain genes and other unneeded segments of DNA turned off is to pack them into a highly condensed form of chromatin called "heterochromatin." A potential danger to this action, however, is that the packaging mechanism might reach out too far along a stretch of DNA, grab genes that should be turned on, and bury them in heterochromatin. Just beyond the beta-globin locus, in fact, is a segment of heterochromatin. An insulator element positioned between these regions of DNA acts as a barrier to keep the heterochromatin from propagating too far, and thus protects the beta-globin locus from being silenced. Recently, the scientists found that part of this insulator recruits certain proteins that chemically modify histones in a way that may halt the formation of heterochromatin. This part of the insulator is distinct from the portion that works to block nearby enhancers. Continued study of insulators and chromatin structure will lead to further insights into the regulation of genes, a process critical for health and development.

Felsenfeld G, Burgess-Beusse B, Farrell C, Gaszner M, Ghirlando R, Huang S, Jin C, Litt M, Magdinier F, Mutskov V, Nakatani Y, Tagami H, West A, and Yusufzai T. Chromatin Boundaries and Chromatin Domains. Cold Spring Harbor Symposia on Quantitative Biology, Symposium 69, Pages 1-6, 2004.

West AG, Huang S, Gaszner M, Litt MD, and Felsenfeld G. Recruitment of histone modifications by USF proteins at a vertebrate barrier element. *Mol Cell* 16: 453-463, 2004.

FEATURE

Insights into Cellular Communication Through Medicinal Chemistry

Although a cup of coffee first thing in the morning is used as an eye-opener by millions, not many people know precisely why or how the caffeine in coffee wakes them up. In fact, a closer look at the molecular actions of caffeine offers a tantalizing peek into a world of complex, interconnected molecular signals. Understanding such signaling processes may hold the key to new treatments for diseases as diverse as asthma, cystic fibrosis, stroke, cardiovascular disease, and glaucoma. Caffeine acts on adenosine receptors, and the molecule that naturally occupies this receptor—and the pathways it activates—are the subject of an ambitious research program in the NIDDK intramural Molecular Recognition Section. This Section, headed by Dr. Ken Jacobson, works at the cutting edge of synthetic organic chemistry and molecular modeling to perform studies of the fundamental nature of molecular signaling through receptors for adenosine. Through studies of "medicinal chemistry," these scientists are making discoveries that may allow physicians in the future to target treatments for a variety of diseases that will be far more effective and have fewer side effects than ever before.

Connection between Caffeine, Adenosine, and Metabolism

Nearly twenty years ago, researchers identified the biochemical mechanism that explains just how that cup of coffee first thing in the morning works. They identified the molecular receptor on the surface of cells to which caffeine binds. When this naturally-occurring stimulant found in coffee, tea, and chocolate binds to this receptor, it prevents the molecule that naturally occupies this site, adenosine, from binding. Adenosine acts a natural depressant; by displacing it, caffeine removes a "brake."

With adenosine out of the way, cellular activity increases, nerve cells in the brain begin to fire, blood vessels dilate, the heart beats faster, blood pressure rises, and the liver releases sugar into the bloodstream, producing the "buzz" familiar to millions of coffee drinkers. As the caffeine is slowly metabolized, adenosine re-occupies its receptors, and these physiologic changes slowly reverse. This cycle of metabolic highs and lows helps explain why caffeine is the most widely-consumed mood-altering drug in the world.

Adenosine is a relatively simple, nitrogen- and sugar-containing molecule found throughout the body. It is one of the four components that comprise DNA. It is also the core of adenosine triphosphate, or ATP, a molecular form of energy currency that the cells of the body use to store and release energy. Various forms of adenosine therefore play important roles in information storage (in DNA) and cellular energetics (as ATP and its metabolites). Unmodified adenosine itself also plays an important role in intra- and intercellular signaling and metabolism as well.

Biology of Adenosine and Its Receptors

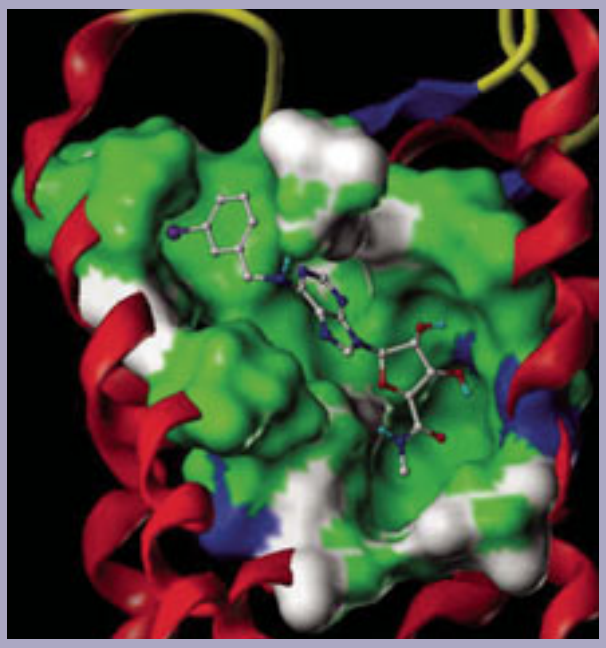
One way cells communicate with one another is through the secretion of small molecules including proteins, carbohydrates, lipids, or nucleosides such as adenosine. These molecules are synthesized and released by cells of almost all types. They may remain in the vicinity of the cell from which they are secreted and have an impact on neighboring cells, or they may travel through the blood stream and have effects on tissues far away.

In order for a cell to respond to a given signaling molecule, it must possess the appropriate receptor to detect it. Once the signaling molecule, or "ligand," binds its

receptor, the receptor undergoes some kind of change. It may change shape, dissociate itself from some proteins and associate itself with new ones, modify other molecules, or perform a host of other possible actions that "tell" the cell that the ligand is present. This change initiates a cascade of events that results in a cellular response—cell division, specialization, or even death, depending on the ligands, receptors, and cell types involved. Ligand/receptor interactions can be fantastically complex. A given ligand may bind more than one kind of receptor. A given receptor may bind more than one kind of ligand. Different receptors may interact with one another. Different cell types may respond differently to the same ligand. The panoply of activity reflects the enormous complexity and sophistication of cellular communications and responses.

The structure of many receptors is fairly straightforward. In a basically linear chain of amino acids, one end of the receptor sticks outside the cell, a short central region spans the membrane, and the other end of the receptor is inside the cell. Receptors for adenosine, in contrast, are members of the seven transmembrane (7TM) receptor family, so-called because these receptors cross back and forth through the cell membrane a total of seven times. In humans, the 7TM receptor family is believed to contain at least 600 members that are found in a wide range of cells involved in processes as diverse as sight, smell, nerve signaling, and regulation of hormones.

Adenosine signaling plays an important role in a number of crucial physiologic processes, including the proper functioning of the cardiovascular system. In the heart, under normal conditions, the adenosine-containing ATP is broken down to provide energy to power the life-sustaining contractions of cardiac muscle. However, during periods of prolonged elevated heart rate, ATP may be metabolized into a free form, which can then exit the cells. Once outside the cells, adenosine binds to its receptor on the surface of the heart cells, and acts to naturally slow the contractions of the muscle. This feedback loop protects the cardiac muscle against damage that could arise from chronic overstimulation of the heart.



Dr. Ken Jacobson and the researchers of NIDDK's intramural Molecular Recognition Section are using molecules that can bind to and activate (or inhibit) human proteins called adenosine receptors to study receptor signaling. Shown is a computer-generated model illustrating how one such activating molecule (an "agonist") binds to one subtype of adenosine receptor. This image, a closer view of the larger image shown at the beginning of this chapter, shows the details of the interaction of the agonist with the binding site of the receptor. Image courtesy of Dr. Ken Jacobson, Chief, Molecular Recognition Section, NIDDK Division of Intramural Research.

Adenosine also plays another, perhaps more crucial role in the heart. A heart attack, or myocardial infarction, occurs when one of the arteries supplying blood to the heart muscle is blocked. When cardiac muscle downstream of the blockage is deprived of fresh blood and the oxygen it carries, it is damaged and may die. If the blockage is transient and blood flow is quickly restored, the damage to the heart muscle may be minimized and the heart can continue to beat. However, if the blockage is prolonged and blood is cut off for an extended period of time, the damage may be so great that the organ can no longer function; the heart attack is fatal. Scientists have observed that multiple, brief periods of oxygen deprivation—such as those resulting from transient blockages—have the curious effect of

significantly reducing the damage to cardiac muscle cells caused by a subsequent, lengthier blockage in culture and animal models. Importantly, this protective effect, called "preconditioning," is not seen in experimental models where the binding of adenosine to its receptor is blocked. These finding suggest an important role for adenosine signaling in protecting cardiac muscle from transient oxygen deprivation.

At first glance, it might seem prudent to study whether it is beneficial to give people at risk for a heart attack adenosine itself. The problem with this approach is that there are four related receptor subtypes for adenosine—denoted A_1, A_2A, A_2B, and A_3—and each subtype is capable of eliciting its own unique response. A significant challenge for researchers and clinicians is to identify the relationship between activation of a specific adenosine receptor subtype and a particular cellular response, because perpetually elevated levels of adenosine given to protect the heart could have deleterious effects on other tissues.

The drug theophylline, used widely to treat asthma, illustrates the kind of problems that can arise with nonspecific activation of adenosine receptors. Theophylline has a structure similar to adenosine and relaxes the bronchial tubes in the lungs to ease breathing. It is thought to act through the A_2B adenosine receptor subtype. However, in the kidneys and brain, acting through the A_1 receptor, theophylline works as a diuretic and can cause sleep disruption. These side effects make it a less-than-ideal treatment. A better treatment for asthma would have theophylline's beneficial effect in the lungs, but not its unwanted effects in the kidneys and brain. But how would one go about designing such a drug?

Research at the Interface of Chemistry and Medicine

The NIDDK's Dr. Ken Jacobson studies the structure and function of adenosine and other nucleotide receptors and the ways in which signaling through these receptors might be modulated as therapy. Historically, the use of engineered adenosine receptor activators (agonists) and inhibitors (antagonists), has been constrained in research studies and in the treatment of patients because of limited knowledge of the receptors' structures. Their usefulness has also been limited by the lack of receptor subtype specificity for many potential agonists or antagonists. Dr. Jacobson and his team of scientists in the Molecular Recognition Section have worked to design modified ligands that activate specific adenosine receptor subtypes as part of an overarching investigation into the structure/function relationship that underlies the adenosine/receptor pair. Though primarily used as research tools today, such molecules could one day be used as novel therapies for diseases in which cell signaling through adenosine receptors is disrupted. Such novel therapies could be designed to have greater specificity and fewer side effects than current adenosine receptor-targeting drugs.

A significant impediment to studies of signaling through adenosine receptors is the lack of knowledge regarding their three-dimensional structure; in fact, the detailed structure of most 7TM receptors is largely unknown. The unusual, multiple membrane-spanning stretches of the protein make these molecules ill-suited for methods, such as X-ray crystallography, that have been traditionally used to probe structure/function relationships in biomolecules. In fact, there is only one 7TM receptor for which a crystal structure has been solved—the photoreceptor rhodopsin, which is found in the eye. However, advances in computer technology and computing power have allowed Dr. Jacobson and his team of researchers to use the structure of rhodopsin as a starting point to generate models of 7TM receptors such as those for adenosine. This iterative process involves computer modeling of the interactions between adenosine and its receptors. This information can then guide the chemical and molecular biological synthesis of ligands or receptors with the incorporation of subtle alterations. Experimentally measuring the impact of these alterations on the ligand-receptor interaction and using the data generated in these studies help to further refine the computer model. After multiple rounds, this process results in the emergence of molecules that have been rationally designed to

activate or inactivate a receptor upon binding. These are highly potent, highly specific synthetic agonists or antagonists for a given receptor subtype.

The researchers in the Molecular Recognition Section have used this sophisticated approach to begin to elucidate at the molecular level the nature of the interactions between ligands and receptors. They have designed and successfully tested selective, potent agonists or antagonists for all four subtypes of adenosine receptors. Many of these molecules are especially valuable research tools because they are effective in cells obtained from many species. Thus, researchers working with cells derived from mouse, rat, rabbit, cow, or humans can all use these molecules in their studies.

The development of subtype-specific adenosine receptor agonists and antagonists has led Dr. Jacobson's team into a number of fruitful collaborations with scientists external to the NIDDK. Working with researchers at the University of Connecticut Health Center, Dr. Jacobson's group explored the molecular basis of the cardioprotective effective of adenosine described earlier. Using cultured chick cells, the scientists found that specific agonists of the A_1 receptor subtype offered short-term protection against oxygen deprivation, while agonists specific for the adenosine A_3 receptor subtype offered long-term protection. However, when both A_1 and A_3 agonists were present, an additional protective effect was seen. This finding could lead to the development of adenosine receptor agonists specifically tailored to have a protective effect in people at risk of a heart attack and simultaneously avoid the problems associated with less-specific agonists.

Future of Adenosine and Adenosine Receptor Engineering

Dr. Jacobson's future research goals are far more ambitious than devising new ways to activate nucleoside receptor subtypes. Dr. Jacobson, his research team, and his collaborators are hard at work exploring the possibility of synthesizing unique, customized ligand-receptor pairs to facilitate targeted cell signaling. Properly designed, each customized ligand would interact only with its customized receptor, and *vice versa*. These molecules, called "neoceptors" by Dr. Jacobson, would largely avoid the problems associated both with receptors that bind multiple ligands and with ligands that bind multiple receptor subtypes. Such an approach could also minimize the likelihood of unwanted side effects from drug treatment, because the only tissue or organ that would be capable of responding to the customized ligand would be the one(s) bearing the customized neoceptor. This pioneering work, still in its earliest stages, will need further refinement before it is ready to move out of the laboratory and into the clinical setting. However, it has shown promise in some preliminary studies in cultured cells. Using this approach, Dr. Jacobson and his team have provided a cardioprotective effect in heart cells similar to that seen with selective adenosine receptor agonists. Translation of these laboratory discoveries into clinical research applications is an ultimate goal of this research.

Currently, there is no reliable way to deliver these neoceptors in a targeted fashion in humans, so therapies based on neoceptors are years away. To be successful, this approach will require advances in targeted gene therapy in order to deliver the neoceptors to their target tissue, so that they can signal the presence of their customized ligand. Nevertheless, this novel, highly exploratory research by the NIDDK sets the stage for rational drug development by providing the tools and raw materials for future studies. It represents an excellent example of how the most fundamental studies can plant the seeds for future treatment strategies. As a whole, Dr. Jacobson's research illustrates how something as ordinary and widely-consumed as caffeine can lead to insights into molecular signaling, how research into the pathways influenced by caffeine can open the door to treatments for a number of devastating and costly diseases, and how creative minds can use these insights to fashion new therapies that may one day permit the ultimate in customization of drugs and their targets.

Think about that over your next cup of coffee.

The NIH-sponsored Diabetes Prevention Program clinical trial demonstrated that through modest weight loss and exercise, people at risk of developing type 2 diabetes can delay or prevent its onset. The "Small Steps. Big Rewards. Prevent Type 2 Diabetes" campaign spreads this important prevention message of hope. The campaign is led by the National Diabetes Education Program (NDEP), which is a partnership of the NIDDK of the NIH, the CDC, and over 200 public and private organizations. The NDEP has created tailored materials and messages for audiences at high risk of developing type 2 diabetes, including African Americans, Hispanic and Latino Americans, American Indians and Alaska Natives, Asian Americans and Pacific Islanders, and older adults. Campaign information and materials can be found at *http://ndep.nih.gov/campaigns/SmallSteps/SmallSteps_index.htm*.

Diabetes, Endocrinology, and Metabolic Diseases

NIDDK support of basic and clinical research in the areas of diabetes, endocrinology, and metabolic diseases spans a vast and diverse range of diseases and conditions, including diabetes, osteoporosis, cystic fibrosis, and obesity. Together, they affect many millions of Americans and profoundly decrease their quality-of-life. Many of these diseases are complex—an interplay between genetic and environmental factors contributes to disease development.

Diabetes is a debilitating disease that affects an estimated 18.2 million people in the U.S.—over 6 percent of the total population—and is the sixth leading cause of death. Diabetes lowers average life expectancy by up to 15 years, increases cardiovascular disease risk two-to-four-fold, and is the leading cause of kidney failure, lower limb amputations, and adult-onset blindness. In addition to these human costs, the estimated total financial cost for diabetes in the U.S. in 2002—including costs of medical care, disability, and premature death—was $132 billion. Effective therapy can prevent or delay these complications, but approximately one third of Americans with diabetes are undiagnosed.

Diabetes is characterized by the body's inability to produce and/or respond appropriately to insulin, a hormone which is necessary for the body to absorb and use glucose (sugar) as a cellular fuel. These defects result in persistent elevation of blood glucose levels and other metabolic abnormalities, which in turn lead to the development of disease complications. The most common forms of diabetes are type 1 diabetes, in which the body completely loses its ability to produce insulin; and type 2 diabetes, in which the body becomes resistant to insulin signaling, and which can eventually result in impaired insulin production.

Type 1 diabetes affects approximately 5 to 10 percent of individuals with diagnosed diabetes. It most often occurs in children, but may appear at any age. Type 1 diabetes is an autoimmune disease, in which the immune system mistakenly attacks and destroys the beta cells of the pancreas. These beta cells, which are found within tiny cell clusters called islets, are the body's sole producers of insulin. If left untreated, type 1 diabetes results in death from starvation despite high levels of glucose in the bloodstream. Thus, patients require lifelong insulin administration—in the form of multiple daily injections or via an insulin pump—in order to regulate their blood glucose levels. Despite vigilance in disease management, with frequent finger sticks to test blood glucose levels and the administration of insulin, it is still impossible for patients to control blood glucose levels as well as they could if they had functional beta cells. Thus, researchers are actively seeking new methods to improve blood glucose monitoring and insulin delivery, as well as working on new beta cell replacement therapies meant to cure type 1 diabetes.

Type 2 diabetes is the most common form of the disease, accounting for up to 95 percent of diabetes cases in the U.S. Type 2 diabetes is associated with several factors, including older age and a family history of diabetes. It is also strongly associated with obesity: more than 80 percent of people with type 2 diabetes are overweight or obese. Type 2 diabetes occurs more frequently among minority groups, including African Americans, Hispanic Americans, American Indians, and Native Hawaiians.

In patients with type 2 diabetes, cells in muscle, fat, and liver tissue do not properly respond to insulin. Gradually, the pancreatic beta cells secrete less and less insulin, and the timing of insulin secretion becomes abnormal. To control glucose levels, treatment approaches include diet, exercise, and medications; some patients also need to take insulin. There are also millions of individuals who have a condition called "pre-diabetes," in which blood sugar levels are higher than normal, but not as high as in diabetes. This population is at high risk of developing diabetes. Fortunately, the Diabetes Prevention Program (DPP) clinical trial has shown that patients with pre-diabetes can dramatically reduce their risk of developing full-blown diabetes with improvements in lifestyle or with drug treatment.

Type 2 diabetes was previously called "adult-onset" diabetes because it was predominantly diagnosed in older individuals. However, this form of diabetes is increasingly being diagnosed in children and adolescents, and it disproportionately affects minority youth. Believed to be related to increasing rates of pediatric obesity, this is an alarming trend for many reasons. First, the onset and severity of disease complications correlate with the duration of diabetes; thus, those with early disease onset are at greater risk with respect to complications. Second, maternal diabetes during pregnancy—either onset of type 2 diabetes before pregnancy or the development of gestational diabetes during pregnancy—confers an increased risk of diabetes in offspring. Thus, the rising rates of diabetes and pre-diabetes in young women could lead to a vicious cycle of ever-growing rates of diabetes. Third, diabetes often becomes more difficult to control over time. With longer duration of disease, health care providers may find it increasingly difficult to strictly control a patient's blood sugar and thus prevent or delay the development of complications. Therefore, the advent of type 2 diabetes in youth has the potential to drastically worsen the enormous health burden that diabetes already places on the U.S.

The NIDDK is supporting research to better understand the mechanisms that lead to the development and progression of diabetes and the many other endocrine and metabolic diseases within the Institute's mission; such research will ultimately spur the design of potential new intervention strategies. In parallel, based on knowledge from past scientific research investments, the Institute is vigorously pursuing studies of prevention and treatment approaches for these diseases.

ADVANCES AND OPPORTUNITIES IN DIABETES RESEARCH

New Imaging Technology To Monitor Type 1 Diabetes Disease Progression: Type 1 diabetes is usually diagnosed very late in disease progression, when most of the beta cells of the pancreas (which produce insulin) have already been destroyed by an autoimmune attack. Currently, there is no way to detect the first signs of beta cell destruction to monitor disease progression. In research toward overcoming this major research and clinical barrier, scientists discovered a new, non-invasive imaging technology that enabled them to monitor disease progression in a mouse model. The technology uses a vascular probe, containing magnetic nanoparticles that can be detected by magnetic resonance imaging (MRI). If type 1 diabetes has already begun to develop, the probe leaks out of the blood vessels of the pancreas and can be visualized by MRI. Vascular probes have already been successfully used in humans to detect prostate cancer metastases; therefore, this technology has high potential of being translated to the clinic for type 1 diabetes. Importantly, this technology can facilitate studies of the molecular underpinnings of disease onset and progression, which can lead to novel prevention and treatment strategies.

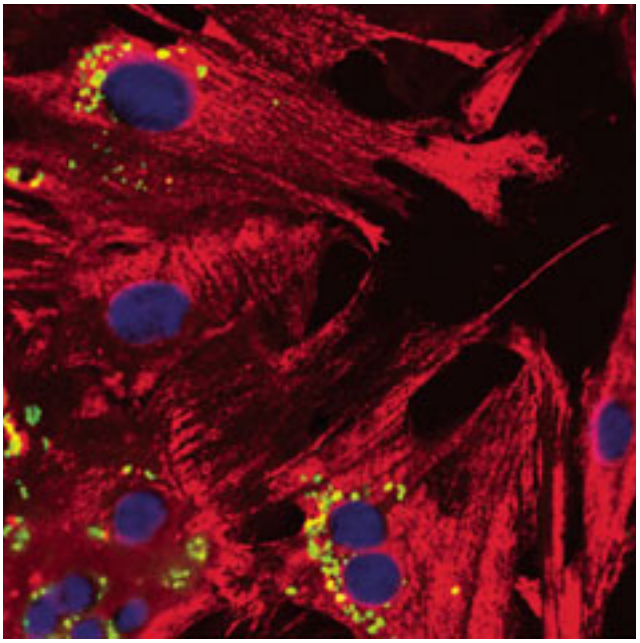
Denis MC, Mahmood U, Benoist C, Mathis D, and Weissleder R. Imaging inflammation of the pancreatic islets in type 1 diabetes. *Proc Natl Acad Sci USA* 101: 12634-12639, 2004.

Potential Source of Islet Cells for Future Cell Therapies: Research is helping to build understanding of beta cell regeneration, with implications for potential diabetes treatments. Scientists at the NIDDK's intramural laboratories have induced human insulin-producing cells to revert to islet precursor cells, proliferate, and then differentiate into islet-like cells again. The researchers first removed islets from human cadaver pancreata, and exposed these islets to a medium containing animal serum. Over time, cells migrated out until the original islets were depleted. These migrating islet cells, identified as insulin-expressing cells, then turned into more primitive precursor cells that do not produce insulin. These new cells, called human islet-derived precursor cells, reproduce easily to form many more cells. They also appear to naturally and efficiently differentiate into clusters of islet-like cells when subsequently exposed to a serum-free medium. The differentiated cells produce much less insulin than the original cells, but do show many of the characteristics of the original beta cells. While these cells appear to be different from stem cells, the scientists noted that their studies do not preclude the possibility that adult islet stem cells may exist. For the future, the researchers hope to define the optimal environmental conditions to grow precursor cells and to stimulate them to differentiate into hormone-producing cells. Their goal is to design a cellular environment as close as possible to the natural environment of a healthy human pancreas. Another challenge is to develop a culture medium that does not rely on animal serum, so cells grown in the laboratory could be transplanted back into people with a minimum risk of side effects. Because of the relatively small number of cadaveric donor pancreata available for transplantation, research toward developing new sources of islet cells is critical for future therapeutic use.

Gershengorn MC, Hardikar AA, Wei C, Geras-Raaka E, Marcus-Samuels B, and Raaka BM. Epithelial-to-Mesenchymal Transition Generates Proliferative Human Islet Precursor Cells. *Science* 306: 2261-2264, 2004.

Defects in the Cell's Energy-converting Machines, Mitochondria, Are Linked to Type 2 Diabetes and Cardiovascular Disease Risk Factors: Mitochondria are components of cells that can extract energy from molecules derived from food and convert it into a form that is used to fuel the cell's biological processes; in certain cells, the mitochondria dissipate energy from food sources as heat, rather than storing it in a form for future use. While some components of mitochondria are encoded by genes in the cells' nucleus, where most of the cell's genetic material resides, mitochondria also harbor their own separate genomes. Researchers exploring the underpinnings of diabetes and other metabolic problems have recently identified mitochondrial defects as potential contributing factors to these health conditions.

To improve medical strategies for the prevention of diabetes, it is important to know the precise mechanisms underlying disease development. While obesity is a serious risk factor for type 2 diabetes, it does not fully explain the disease, because many obese people are not diabetic, and some people of normal weight develop diabetes. Identification of the biologic basis for diabetes susceptibility is key to development of new therapies. Several recent lines of evidence suggest that people with type 2 diabetes may have defects in the functioning of mitochondria, the structures in cells responsible for converting fat into useful energy. A new study reports that these defects precede the development of the disease: people at risk for type 2 diabetes accumulate fats in muscle cells, and this accumulation correlates with mitochondrial problems. Because the presence of such fats has been shown in experimental models to decrease the ability of cells to function properly in response to insulin, deficits in mitochondrial function could potentially contribute to the insulin-resistance that can lead to type 2 diabetes. These insights may help pave the way to the development of therapies aimed at correcting mitochondrial function as a possible means of preventing or delaying onset of the disease.



In laboratory studies, scientists at the NIDDK have induced insulin-producing cells, obtained from human pancreatic tissue, to revert to islet precursor cells. These precursor cells are capable of expansion and appear to naturally and efficiently differentiate into clusters of islet-like cells. The image shows cells during a laboratory experiment in which insulin-expressing human cells were induced to form islet precursor cells. This work may help to clarify the natural lifecycle of the beta cell and may eventually have applications for diabetes treatment. Photo courtesy of Dr. Marvin C. Gershengorn, NIDDK, and reprinted from Gershengorn et al. *Science*, online publication 25 November 2004, 10.1126/science.1101968; print: *Science* 306: 2261-4.

Another group of scientists sought to discover genetic factors that may contribute to a clustering of certain metabolic defects by studying a large family in which many members suffered from these conditions. The metabolic defects included hypertension and abnormal blood lipids (fat molecules)—conditions that often occur together for reasons that have not been clear—as well as defects in levels of blood magnesium. The scientists traced these medical conditions in the large family to a mutation in the mitochondrial genome. The results of this study may direct future investigations of potential mitochondrial defects as contributing to the clustering of hypertension, blood lipid abnormalities, and other metabolic problems commonly seen in the population.

Petersen KF, Dufour S, Befroy D, Garcia R, and Shulman GI. Impaired mitochondrial activity in the insulin-resistant offspring of patients with type 2 diabetes. *N Engl J Med* 350: 664-671, 2004.

Wilson FH, Hariri A, Farhi A, Zhao H, Petersen KF, Toka HR, Nelson-Williams C, Raja KM, Kashgarian M, Shulman GI, Scheinman SJ, and Lifton RP. A Cluster of Metabolic Defects Caused by Mutation in a Mitochondrial tRNA. *Science* 306: 1190-1194, 2004.

Patient Literacy Affects Success of Type 2 Diabetes Disease Management: Patients with diabetes can minimize complications by reducing the level of sugar in their blood. While many diabetes disease management programs have helped patients reduce their blood sugar levels by using a combination of education, medication, diet and exercise regimens, and glucose monitoring, their use in socially disadvantaged populations has been less successful. Low literacy is common among patients and is associated with poor knowledge about diabetes. A recent study examined the role of literacy on the effectiveness of a comprehensive disease management program for patients with type 2 diabetes. In the study, half of the 217 patients received usual care from their primary care clinician, while the rest received usual care plus supplemental intensive diabetes management that included one-on-one counseling and medication management. The individualized care included tools to enhance comprehension such as simplified verbal explanations, picture-based materials and "teach-back" patient comprehension assessments. The supplemental intervention significantly improved the blood sugar control in patients with low literacy (below sixth-grade level). Patients with higher literacy showed improvement from the usual care regardless of whether or not they also received the individualized care. These results suggest that providing individualized care can improve the success of diabetes management, and that patients with low literacy stand to benefit the most from such care.

Rothman RL, DeWalt DA, Malone R, Bryant B, Shintani A, Crigler B, Weinberger M, and Pignone M. Influence of patient literacy on the effectiveness of a primary care-based diabetes disease management program. *JAMA* 292: 1711-1716, 2004.

Examples of New NIDDK Clinical Research Efforts on Type 1 Diabetes: The NIDDK is pursuing a variety of avenues of clinical research on type 1 diabetes. Several new studies are being conducted by Type 1 Diabetes TrialNet. TrialNet is an international network of investigators, clinical centers, and core support facilities whose aim is to recruit patients and support studies that will result in an improved understanding of type 1 diabetes disease development and will test strategies for its prevention. TrialNet is spearheaded by the NIDDK and is co-sponsored by the National Institute of Allergy and Infectious Diseases (NIAID), the National Institute of Child Health and Human Development (NICHD), and the Juvenile Diabetes Research Foundation International (JDRF). The TrialNet's Type 1 Diabetes Natural History Study will probe the causes of type 1 diabetes by examining the immune and metabolic events leading to disease onset in individuals who are at-risk for disease development. TrialNet also recently launched a study to test two immunosuppressive agents—mycophenolate mofetil and daclizumab—to determine if they are able to safely delay or stop the immune destruction of remaining beta cells in new-onset type 1 diabetes patients. Another new TrialNet effort is a pilot clinical study based on observations from epidemiologic studies that children who have received omega-3 fatty acid—either in the womb or during the first year of life—have a lower risk of developing type 1 diabetes. Epidemiologic studies are useful for generating hypotheses to be tested in randomized clinical trials. The new pilot clinical study will assess the feasibility of a large scale trial to determine whether nutritional supplements with an omega-3 fatty acid will prevent the development of islet autoimmunity.

The NIDDK, in another international effort, also in partnership with NIAID, NICHD and JDRF as well as the National Institute of Environmental Health Sciences and the CDC, has begun recruitment for a study that will seek to identify infectious agents, dietary factors, and/or other potential environmental conditions that might trigger type 1 diabetes in genetically susceptible newborns. This study is called The Environmental Determinants of Diabetes in the Young (TEDDY).

The NIDDK, in collaboration with the National Institute of Allergy and Infectious Diseases, has also recently funded a new Clinical Islet Transplant (CIT) consortium. The consortium's studies will focus on improving the safety and long-term success of methods for transplanting islets in people with type 1 diabetes.

The Type 1 Diabetes - Rapid Access to Intervention Development (T1D-RAID) is a cooperative program of the NIDDK and the National Cancer Institute designed to facilitate translation to the clinic of novel, scientifically meritorious therapeutic interventions. It will do this by making available, on a competitive basis, NCI resources for the pre-clinical development of drugs, natural products, and biologics. A partial listing of those services includes: high-throughput screening, studies in animal models, formulation, pharmacology and toxicology studies, and bulk substances acquisition. T1D-RAID is intended to remove the most common barriers between laboratory discoveries and clinical trials of new molecular entities. The goal of T1D-RAID is to support the preclinical work needed for the clinical "proof of principle," which is the study that will determine if a new molecule or novel approach is a viable candidate for expanded clinical evaluation.

Finally, the NIDDK has expanded its Web pages on the Special Statutory Funding Program for Type 1 Diabetes Research, which it administers on behalf of the Secretary, HHS, and in which multiple NIH components and the CDC participate. These Web pages include resources for investigators, such as funding opportunities and availability of tools and materials for research, as well as information for patients and family members on clinical studies in which they may wish to participate.

Building on the Success of the Diabetes Prevention Program (DPP) Clinical Trial: The NIDDK is pursuing several efforts to build upon the findings of the landmark NIH-sponsored Diabetes Prevention Program (DPP) clinical trial. The DPP examined the effects of lifestyle and medical interventions on the development of type 2 diabetes in adults at risk

for this disease. Upon entering the trial, the over 3,000 participants had elevated blood glucose levels and were overweight, and thus were at substantial risk for developing type 2 diabetes. Nearly one-half of the participants were from minority groups, and approximately two-thirds were women. The lifestyle intervention included modest weight loss and exercise. The results of the lifestyle intervention demonstrated a dramatically reduced risk—by 58 percent—of developing type 2 diabetes in a population at high risk. The medication intervention using the drug metformin reduced diabetes risk by 31 percent. The lifestyle and metformin interventions worked well in both men and women and in all ethnic groups studied; the lifestyle intervention was also particularly effective in older participants.

"Small Steps. Big Rewards. Prevent Type 2 Diabetes"—Importantly, the dramatic health benefit resulting from the lifestyle intervention of DPP required only modest weight loss and exercise. This concept is now part of a new educational campaign to promote the findings of the DPP, called "Small Steps. Big Rewards. Prevent Type 2 Diabetes." The campaign is led by the National Diabetes Education Program (NDEP), which is a partnership of the NIDDK of the NIH, the CDC, and over 200 public and private organizations. The NDEP has created tailored campaign messages and materials for high risk audiences: African Americans, Hispanic and Latino Americans, American Indians and Alaska Natives, Asian Americans and Pacific Islanders, and older adults, as well as materials for a general audience. In addition, the NDEP and its partners are promoting diabetes prevention to health care providers to give them the information and tools to help their patients take small but important steps to prevent the disease. Additional information on the "Small Steps. Big Rewards. Prevent Type 2 Diabetes" campaign is presented in the sidebar on the NDEP, in this chapter. Information and campaign materials are also available at: *http://ndep.nih.gov/campaigns/SmallSteps/SmallSteps_index.htm.*

Assessing the Durability of the DPP Interventions: The DPP Outcomes Study (DPPOS)—The DPPOS is a follow-up study of participants in the DPP clinical trial. The DPPOS will examine the durability of the DPP interventions on prevention or delay of type 2 diabetes and its cardiovascular complications; heart disease is the major cause of death in people with type 2 diabetes. The DPPOS will additionally examine the ability to maintain weight loss in the participants over extended periods of time. The study will also investigate other associated health conditions in the participants, including, for example, kidney disease and urinary incontinence.

Research Demonstration and Dissemination Projects: Bringing the DPP Results to Patients—The NIDDK is supporting studies to test and evaluate interventions and activities that lead to the application of existing knowledge to disease control and prevention. Among these studies are research projects aimed toward improving translation—in this case, from clinical trial to community patient care—of the results of the DPP. For example, in one study, working with families in whom at least one member has type 2 diabetes, researchers are testing a "family visit program" to help all family members learn how they can adopt healthy lifestyles and better use healthcare and other community resources. The study is being conducted in an area with a substantial Hispanic population. Examples of other translational research projects include studies of a primary care and Web-based intervention for adolescents at risk for diabetes, an intervention that targets couples in which one spouse has type 2 diabetes, and a community-based, family-oriented health program to decrease obesity and risk of type 2 diabetes in children from a high risk, inner-city African American population.

THYROID HORMONE DISORDERS

Thyroid Hormone Requirements During Pregnancy: Thyroid hormone (TH) plays an important role in promoting normal fetal development during pregnancy. When maternal TH levels are too low (hypothyroidism) or too high (hyperthyroidism), the result could be increased fetal mortality or other fetal developmental problems. Hypothyroidism is treated with a synthetic form of TH, called

levothyroxine. Pregnancy increases the requirement for TH, so the dose of levothyroxine in women with hypothyroidism is increased during pregnancy, usually after the first prenatal doctor's visit at approximately 10 weeks of gestation. However, it is unclear if this timing is sufficient to protect the fetus from harmful effects of low TH levels. To learn the timing pattern of TH requirement during pregnancy, researchers studied 19 women who had hypothyroidism and desired pregnancy. They determined that the requirement for increased levothyroxine occurs very early in pregnancy—as early as the fifth week of gestation. Based on these novel observations, the researchers recommend that women with hypothyroidism be counseled before pregnancy to increase their levothyroxine dose immediately upon confirming pregnancy, even before their first prenatal doctor's visit. Another study investigated the opposite situation—the effects of high TH levels on the developing fetus. Researchers studied individuals who have "resistance" to thyroid hormone (RTH), and whose thyroid produces very high levels of TH in compensation. Because mothers with RTH make high levels of TH during pregnancy, the researchers could investigate the effects of high TH levels on the fetus. The researchers observed a 3- to 4-fold increase in the rate of miscarriage in the women with RTH. In addition, they observed differences in birth weights of the babies born to women with RTH. When the newborn also had RTH, the birth weight was normal; however, if the newborn did not have RTH, the birth weight was low. These results suggest that high levels of TH could be damaging to the fetus and result in increased rates of miscarriage and low birth weights. Thyroid disorders are prevalent in women and TH is one of the most commonly prescribed medications. Taken together, these studies emphasize the importance of maintaining normal TH levels during pregnancy and suggest adjustment of TH medicines earlier in pregnancy than is the current practice.

Alexander EK, Marqusee E, Lawrence J, Jarolim P, Fischer GA, and Larsen PR. Timing and magnitude of increases in levothyroxine requirements during pregnancy in women with hypothyroidism. *N Engl J Med* 351: 241-249, 2004.

Anselmo J, Cao D, Karrison T, Weiss RE, and Refetoff S. Fetal loss associated with excess thyroid hormone exposure. *JAMA* 292: 691-695, 2004.

CYSTIC FIBROSIS

Curcumin as a Potential Treatment for Cystic Fibrosis: Cystic fibrosis (CF) is a genetic disorder that results in the accumulation of thick, sticky mucus in the lungs, causing damage and facilitating infections. CF is caused by mutations in the gene encoding the CFTR protein, which resides in the outer surface of cells lining such tissues as the lung and intestine, where it regulates the movement of chloride. The most common mutation of the gene, ΔF508, yields a protein that would be functional, but which is degraded before it reaches the cell surface. Researchers have recently tested the effect of a compound called curcumin, purified from the spice turmeric, in a mouse model of CF. When given to mice that are genetically engineered to have the ΔF508 mutation, curcumin treatment enabled the mutant form of the CFTR protein to function effectively, presumably by allowing it to reach its normal cellular destination. Indeed, when cells cultured from animals with the ΔF508 mutation were treated with curcumin, the protein was properly routed to the cell surface. Importantly, the amount of curcumin that achieved these promising results in mice is equivalent to a dose that has been well-tolerated by humans in previous studies. Therefore, curcumin, which is already known to be safe in people, has the potential to be of value for patients with this devastating illness.

Egan ME, Pearson M, Weiner SA, Rajendran V, Rubin D, Glöckner-Pagel J, Canny S, Du K, Lukacs GL, and Caplan MJ. Curcumin, a major constituent of turmeric, corrects cystic fibrosis defects. *Science* 304: 600-602, 2004.

NIDDK AIDS RESEARCH

Research supported by the NIDDK has contributed to the current understanding of AIDS wasting syndrome. With the widespread adoption of highly active antiretroviral therapy (HAART), which has markedly

improved survival, the incidence of AIDS wasting syndrome has declined. Unfortunately, HAART and HIV infection are associated with a variety of metabolic complications, collectively termed "lipodystrophy syndrome." This syndrome may include abnormal distribution of body fat, dyslipidemia (elevated levels of unhealthy fats in the blood) and insulin resistance. These metabolic abnormalities are major risk factors for the development of serious diseases, such as diabetes and cardiovascular disease. The NIDDK supports a number of research studies aimed at understanding the causes and exploring possible therapies for HIV-associated lipodystrophy.

Hormonal Treatment for HIV-infected Men with Lipodystrophy: Some HIV-positive men with lipodystrophy and excess abdominal fat have reduced levels of growth hormone (GH). Restoring GH to normal levels is a potentially attractive approach to treating these individuals, as GH has been shown to reduce visceral fat in GH-deficient patients. Unfortunately, high-dose GH therapy can result in insulin resistance and other complications. An alternative strategy to normalize GH levels is to provide an agent that promotes increased secretion of GH by the pituitary gland, such as growth hormone-releasing hormone (GHRH). Researchers recently compared GHRH therapy with placebo (sugar pill) in 31 HIV positive men with lipodystrophy over 12 weeks. The effectiveness of the treatment was determined by measuring levels of IGF-1, a protein secreted in response to GH stimulation that mediates many of its actions. GHRH therapy significantly increased levels of IGF-1 in treated individuals, and was associated with significant improvements in a number of body mass parameters, including increased lean body mass, decreased trunk fat, and reduced abdominal visceral fat. Levels of blood glucose, insulin, and lipids did not change significantly. GHRH therapy, which is aimed at returning GH to a more normal range, may be beneficial in HIV positive individuals with diminished levels of the hormone. GH concentration might not reach harmful levels during treatment with GHRH because other factors that are also influenced by this hormone are present to provide feedback into GH production, if it becomes too high. GHRH therapy may therefore represent a more "natural" way of restoring GH levels to the normal range.

Koutkia P, Canavan B, Breu J, Torriani M, Kissko J, and Grinspoon S. Growth hormone-releasing hormone in HIV-infected men with lipodystrophy: a randomized controlled trial. *JAMA* 292: 210-218, 2004.

Liver disease is an important cause of sickness and death in persons infected with HIV. HIV-infected persons, like non-HIV infected persons, can develop hepatitis B and C, NASH, alcoholic liver disease, drug-induced liver disease, and opportunistic infections of the liver and biliary tree. In HIV-infected persons receiving HAART, liver disease caused by chronic viral hepatitis has emerged as a leading cause of death, due in large part to hepatitis C virus (HCV) co-infection, a consequence of a shared transmission route for the two viruses. HCV infection is adversely affected by co-infection with HIV at every stage of its natural history; the proportion of patients who recover is much lower, and the disease progresses from persistent infection to cirrhosis to end-stage liver disease more rapidly. As therapies for HIV have improved and survival has been extended by antiretroviral therapy, liver disease has become a critical problem among HIV-infected persons. The major goals for NIDDK research in HIV and liver disease are to define the causes of liver disease associated with HIV, including interactions between HIV and hepatitis viruses, and to develop means to prevent and treat liver disease in HIV-infected people.

The NIDDK also supports a productive structural biology program within the Institute itself. These projects seek to determine the structures of biologically significant proteins relative to HIV infection, replication, and integration. Through a greater understanding of these structures, the underlying mechanisms of HIV infection are illuminated. Determination of the structure of these proteins is critical for understanding the mode of action of these important molecules and it is also an essential first step in the development of drugs to treat and prevent HIV infection.

Collaborative Islet Transplant Registry – First Report Published

Researchers from 12 medical centers in the United States and Canada, who have performed islet transplants in 86 patients with type 1 diabetes, published their results in the first annual report of the Collaborative Islet Transplant Registry (CITR). The CITR's mission is to expedite progress and promote safety in islet transplantation by collecting, analyzing, and communicating data on this experimental therapeutic procedure. The CITR is supported by a Special Statutory Funding Program for Type 1 Diabetes Research. The report (*www.citregistry.org*) analyzes many factors that can affect the outcome of this experimental procedure for people with severe or complicated type 1 diabetes. It provides data on recipient and donor characteristics, pancreas procurement and islet processing, immunosuppressive medications, function of the donated islets, patients' lab results, and adverse events.

Type 1 diabetes, which affects up to 1 million people in the United States, develops when the body's immune system destroys the insulin-producing beta cells of the pancreas. This form of diabetes usually strikes children and young adults, who need several insulin injections a day or an insulin pump to survive. Insulin, however, is not a cure, and eventually most people with type 1 diabetes develop one or more complications of the disease, including damage to the heart and blood vessels, eyes, nerves, and kidneys. From 1990 to 1999, only 8 percent of islet transplants resulted in insulin independence for more than 1 year. In 2000, however, a group of researchers at the University of Alberta in Edmonton, Canada, reported much greater success in patients transplanted with islets from two to four donor pancreata and treated with an immunosuppressive regimen that left out glucocorticoids, now thought to be toxic to islets. In the next few years, other researchers replicated the "Edmonton protocol" pioneered by the Canadian team, and many centers are now using this approach to islet transplantation.

In islet transplantation, as performed by the 12 participating centers presented in the CITR report, insulin-producing cells derived from donor pancreata were infused into patients with difficult-to-control type 1 diabetes though the portal vein of the liver. When successful, the transplanted islets took up residence in the liver's small blood vessels and began producing insulin. The 86 recipients, who had type 1 diabetes for an average of 30 years, received a total of 158 infusions of islets extracted from 173 donor pancreata. Twenty-eight patients received one islet infusion, 44 received two, and 14 received three. At 6 months after the last infusion, 61 percent of recipients no longer had to inject insulin. At 1 year after the last transfusion, 58 percent were still insulin independent. Some insulin-independent patients, although not receiving insulin, did have higher-than-normal blood glucose levels. Researchers will continue to monitor patients to see how long they remain insulin independent.

Recipients, 66 percent of whom were women, were an average age of 42 years (range 24 to 64 years) and average weight of 143 lbs. (range 103 to 213 lbs.). Before the procedure, nearly half the recipients were using an insulin pump. Most had recently experienced at least one episode of hypoglycemia, or dangerously low blood glucose, requiring another person's help. Their average level of hemoglobin A1c (HbA1c), which reflects blood glucose control over the previous three months, was 7.7 percent, compared to a normal HbA1c of 6 percent.

HbA1c levels generally improved with each infusion, as did levels of fasting blood glucose and C-peptide, which reflect insulin production. One infusion, though rarely providing enough islets to free a person from the need to inject insulin, alleviated episodes of severely low blood glucose. After the first infusion of islets, only two recipients had a low blood sugar problem requiring the

help of another person. None of those who received a single infusion reported a problem with hypoglycemia a year after the procedure.

The centers reported 45 serious adverse events but no deaths in the recipients. The 27 percent of events that were classified as life-threatening included those linked to the transplant procedure itself (e.g., infection, bleeding into the chest or abdomen, low hemoglobin, high liver enzymes) and events linked to medications that suppress the immune system (e.g., anemia, nerve damage, meningitis, and low numbers of white blood cells). Most recipients received the same drug regimen used in the Edmonton protocol: daclizumab at induction to prevent the immune system from rejecting the donor islets and sirolimus, combined with tacrolimus, to maintain immunosuppression.

The CITR is continuing to receive additional data from the inaugural 12 centers and from new centers joining and contributing data. Thus, future reports will be even more comprehensive. Recently, five islet transplant centers in Europe, with funding from the Juvenile Diabetes Research Foundation International (JDRF), began contributing data to the CITR. The CITR is also integrating data from other sources, such as the United Network for Organ Sharing (UNOS) and the Islet Cell Resource Centers. This collaborative effort will provide further critical information on factors that influence the success of islet transplantation.

Because only about 6,000 donor pancreata become available each year, and many are used for whole organ transplantation, the scarcity of islets poses a major obstacle to wider testing of islet transplantation as a treatment for type 1 diabetes. To improve the potential of cell replacement therapy for type 1 diabetes, NIH-funded research is focusing on understanding the beta cell and its regeneration and on efforts to develop alternative sources of beta cells. Researchers are also working on ways to coax the immune system into accepting donated cells or tissues without suppressing the whole immune system.

Hannah Beauregard

The Beauregard Family: What It Is Like to Care for a Young Child with Type 1 Diabetes

The day after two-and-a-half year old Hannah Beauregard had been diagnosed with type 1 diabetes, her parents, Doug and Mary, were being trained at their local hospital by a team of medical personnel on how to measure Hannah's blood glucose level. Blood glucose, or blood sugar, is measured in milligrams per deciliter of blood. Although people with diabetes have higher than normal blood sugar levels, they can also occasionally experience dangerous episodes of seriously low blood sugar. "At one point," Doug recalls, "I told the medical team that I must be doing something wrong because the monitor read 20 (milligrams per deciliter)." The proper target range for Hannah is substantially higher. Before he knew what was happening, attending residents whisked Hannah from his arms and out of her hospital bed into what Doug can only describe as a "little emergency-type" room. "They shut the door and would not allow me in," he vividly recalls.

Hannah Beauregard

What Doug didn't know at the time was that Hannah was being administered a medication that acts like "instant sugar." Because Hannah's blood sugar levels had dropped precipitously, this treatment was necessary to prevent her little body from going into a coma. What Doug did quickly realize was that having a child with diabetes was going to alter life for the Beauregard family dramatically.

"You Are Not Alone"

Doug Beauregard is a third grade teacher and longtime soccer coach. His wife, Mary, is a registered nurse. Given their professions, one would think that they should know a thing or two about children and medical care—and they do, a great deal. But having a young child with type 1 diabetes is often as difficult for them as it is for anyone else. "You're not alone," Doug wrote recently in an email to another parent seeking advice on how to deal with a toddler with type 1 diabetes who was refusing to eat after taking her insulin. "We're facing the same problem with Hannah."

Type 1 diabetes is an autoimmune disease that usually strikes early in life—most patients are diagnosed as children or young adults. Type 1 diabetes destroys the body cells that produce insulin (pancreatic beta cells). Without insulin, the body cannot properly metabolize glucose, a sugar that is the main source of fuel for cells. People with type 1 diabetes must carefully monitor their blood glucose levels throughout the day to determine when they need to eat, and administer insulin, either through injections or an

insulin "pump," to help their bodies use the glucose from carbohydrates in food. Both steps are also necessary to help keep blood sugar levels within a healthy target range. A constant challenge faced by people with type 1 diabetes is matching food intake, physical activity, and insulin doses in order to maintain healthy blood sugar levels; for example, although too little insulin leads to high blood sugar (hyperglycemia), administering too much insulin for the body's needs at a given time can cause blood sugar levels to fall too low (hypoglycemia). Dramatic rises and drops in blood sugar levels can have immediate and life-threatening consequences, and need to be avoided. Moreover, research has shown that carefully controlling blood sugar levels over the long-term is crucial to help prevent serious complications of diabetes, such as diabetic eye, kidney, and nerve disease, and cardiovascular disease.

According to Doug, since November 14, 2002, the day Hannah was diagnosed with type 1 diabetes, he has had only one night of uninterrupted sleep—and that night Doug was sick. "If Hannah snores, whimpers, cries, moves, or whatever, I wake up," he says. He can tell by the way she is sleeping if her blood sugar is low or high. "If I think it is low, I will check her. If not, I try to comfort her."

Doug and Mary love Hannah dearly. Doug, in particular, has made it his mission to tell everyone he can about Hannah and how special she is. "No one is responsible for Hannah's having type 1 diabetes. It's just part of her life, and we love her for who she is," says Doug, who actively tries to help other parents whose children have this life-threatening disease.

Communicating with Others

In many ways, Doug is the consummate communicator. The very first night that Hannah was diagnosed, Doug was on the Internet searching for local support groups. Today, he co-chairs a support group near the family's hometown of Plainwell, Michigan. The group consists of families of children with type 1 diabetes who range in age from 2 to 13 years old. Doug also frequently exchanges emails with people around the world, from Argentina to Newfoundland. "We are all seeking answers for our children," says Doug. "We learn a lot through each other's experiences and mistakes."

The Beauregards' support group meets for discussion and to listen to guest speakers, including representatives from companies who come to explain their product lines for people with diabetes. The group includes a 25-year-old who was diagnosed with type 1 diabetes when she was 15. "Heather describes for us what it was like to be a teenager with diabetes, as well as relates what it's like now to be an active, athletic young woman with the disease," says Mary. Heather is an accomplished volleyball player. Among other things, she serves as a role model for parents in the group who envision their young children as active young adults. Hannah, now 4 years old, takes dance lessons, and is a gymnast, as well as a downhill skier.

But What About All of Those Finger Pricks and Shots?

It is hard enough for adults with type 1 diabetes to take all of the steps necessary to take care of their disease. The question therefore remains, how does a parent convince a small child with type 1 diabetes that enduring finger pricks to test blood glucose levels and shots to administer insulin, several times a day, is necessary in order to stay alive and healthy? And how do parents feel about having to administer those finger pricks and shots?

To help the whole family adjust to Hannah's new health needs, the Beauregards introduced Hannah to a friend—a fluffy brown teddy bear named Rufus. Rufus™, The Bear with Diabetes, was given to Hannah by the organization Childrenwithdiabetes.com. Within hours of their meeting, Rufus became Hannah's fast friend. Rufus is designed so that he, too, needs to have his fingers "pricked" and to be given "shots." It wasn't long before Hannah was administering "shots" to Rufus. After finger pricks to test for glucose levels, both Hannah and Rufus would have their fingers

wiped and a special band-aid applied. When Hannah reminded her bear Rufus that it was time for his evening shot, she was really announcing to her parents that she was ready to have her own shot. The lesson: If Rufus can do it, Hannah can do it, too.

Everyone in Hannah's family—except 10-month-old Evan—knows how to care for her, including her 13-year-old brother, Ryan. "Ryan is really good with his little sister," says Mary. "Yes, they fight and can drive us crazy at times, but Ryan, and everyone on Ryan's soccer team, knows how to test Hannah's blood glucose level," adds Doug.

The good news is that the older Hannah gets, the more choices she can make for herself to help balance her diet, physical activities, and insulin injections so that she can maintain healthy control of her blood sugar levels. As Hannah becomes more independent, the easier it is becoming for her parents. Doug recounted an experience in which he encouraged Hannah in learning about the foods she needs to eat in order to obtain the proper amounts and balance of nutrients she requires at each meal, including carbohydrates. Says Doug, "At dinner the other day, Hannah said she was full. I told her that she needed to eat so she would get her carbs (carbohydrates). Hannah then asked, 'Dad, does my bread have carbs?' Yes, I told her. 'How about my meat?' No, I said. 'I guess I will eat my bread then,' she said." Hannah recognized the need to have her carbohydrates in order to stay healthy. The Beauregards try to make Hannah feel in control of her diabetes as much as possible by giving her choices. "We also always have a fallback food just in case Hannah doesn't want to eat what we have for dinner," Mary adds.

When Hannah reminded her bear Rufus that it was time for his evening shot, she was really announcing to her parents that she was ready to have her own shot. The lesson: If Rufus can do it, Hannah can do it, too.

As much as Doug and Mary sometimes feel they have things pretty much under control, "It's not easy being a parent of a child with diabetes, and it never will be," Doug says. The pre-school Hannah attends, for example, was leery at first about having a student with Hannah's disease, so the Beauregards had to educate the staff about diabetes and what to do if Hannah's blood glucose level is too low or too high. "Part of the problem," says Doug, "is that Hannah isn't always cooperative when her blood glucose level is low." The family has shied away from day care. When Hannah is not at pre-school, Doug's mother, Elizabeth—who is as well-trained as Doug and Mary in how to care for Hannah—spends two or three days a week at the Beauregard home. Doug adds that when he is at work "my students know that if my cell phone rings, it's something important."

In short, life is a constant vigil.

"Because Hannah is doing well, we want to get her story out to people. We feel we have something that we might be able to offer to other parents who are struggling with children who have this disease. It gives us strength."

Hannah is growing up to be an adorable little girl whose life will be in constant jeopardy until a cure is found for her type 1 diabetes. Until then, she will be required to take insulin every day of her life to survive.

"We're not angry that Hannah has (type 1) diabetes," says Doug. He and Mary just want to tell everyone they can about their little girl. "Because Hannah is doing well, we want to get her story out to people. We feel we have something that we might be able to offer to other parents who are struggling with children who have this disease. It gives us strength."

"We need to be strong for every child with diabetes," says Doug, "because without their parents, they won't make it."

PATIENT PROFILE

The NIDDK supports a multi-faceted research program that is investigating ways to help prevent, delay, or possibly cure type 1 diabetes and its complications. For example, the Epidemiology of Diabetes Interventions and Complications (EDIC), is a follow up to an earlier clinical trial in patients with type 1 diabetes. The results of this study continue to demonstrate the importance of beginning, as early as possible, intensive treatment to control blood sugar in order to prevent diabetes-related health complications.

Translating the positive results of research studies into improved care for patients with diabetes is an important aspect of the NIDDK mission. The recently published school guide, "Helping the Student with Diabetes Succeed: A Guide for School Personnel," is a key example of research-based efforts that can contribute to improved care for children with diabetes. This comprehensive guide for managing diabetes in the school setting was developed by the National Diabetes Education Program (NDEP), a collaborative initiative of the NIDDK and the Centers for Disease Control and Prevention. The NDEP uses over 200 public and private partnerships to promote application of research findings that have demonstrated value in the prevention of diabetic complications ensuing from both type 1 and type 2 diabetes, as well as in the prevention of type 2 diabetes. The guide sets out a team approach to diabetes management in schools and outlines the roles and responsibilities of all key school personnel, including school nurses, administrators, teachers, coaches and physical education instructors, bus drivers, lunchroom staff, and guidance counselors, as well as parents and students with diabetes. According to the guide, three key ingredients are needed to ensure successful teamwork:

- *All school staff members who have responsibility for students with diabetes have a basic understanding of the disease and the signs and symptoms of hypoglycemia and hyperglycemia.*
- *The school nurse and/or other trained personnel are available to assist with routine and emergency diabetes care tasks.*
- *Students with diabetes have the ability and are empowered to self-manage their disease as appropriate.*

Copies of the Guide are available for order or download from the NDEP website at http://www.ndep.nih.gov/resources/school.htm

*On the road to improving care for diabetes, the participation of patients in clinical studies is critical. A newly launched website provides patients with type 1 diabetes and their families with information on many of the clinical studies that are seeking volunteers (*http://www.niddk.nih.gov/fund/diabetesspecialfunds/*). This site also features information on research funding opportunities, research resources, and research consortia and networks for investigators studying type 1 diabetes and its complications.*

STOPPing Type 2 Diabetes

Once considered an "adult-onset" disease, type 2 diabetes is being increasingly diagnosed in children and adolescents—especially in minority populations. To address the rising tide of type 2 diabetes in young people, the NIDDK recently launched a new research program, Studies to Treat or Prevent Pediatric Type 2 Diabetes (STOPP-T2D). One facet of this program is a multicenter trial, Treatment Options for Type 2 Diabetes in Adolescents and Youth (TODAY). TODAY is the first NIDDK-sponsored trial to focus on type 2 diabetes in youth. The TODAY trial will compare three treatments for type 2 diabetes in children and teens in 12 medical centers and their affiliated sites across the United States, in order to identify the best therapeutic strategies to combat this disease in young people.

A Rising Tide: Type 2 Diabetes in Youth

About 18.2 million people—6.3 percent of the U.S. population—have diabetes. It is the main cause of kidney failure, lower limb amputations, and new-onset blindness in adults, and is a major cause of heart disease and stroke. Type 2 diabetes, most common in adults over age 40, accounts for up to 95 percent of all diabetes cases. People with type 2 diabetes are impaired in their ability to produce and respond to insulin, a hormone whose proper action is required for the body to absorb and use the sugar glucose as a cellular fuel. The prevalence of type 2 diabetes has risen dramatically in the last 30 years. In the last 10 years alone, the prevalence of diagnosed diabetes cases increased 50 percent, due mostly to the upsurge in obesity in the United States.

However, type 2 diabetes is no longer restricted to adults. Type 2 diabetes has been rising steadily in all children, but especially among African American, Hispanic American and American Indian adolescents, according to reports from clinics around the country. Studies in a number of cities report that childhood type 2 diabetes cases have risen dramatically. By the 1990s, type 2 diabetes accounted for 8 to 45 percent of new childhood diabetes cases, depending on geographic location.

In both adults and children, type 2 diabetes is closely linked to being overweight, inactive, and having a family history of diabetes. According to the 1999 to 2002 National Health and Nutrition Examination Survey (NHANES), about 16 percent of young people ages 6 to 19 are overweight—nearly triple the 1980 rate. Genetic susceptibility, lack of physical activity and unhealthy eating patterns all play important roles in determining a child's weight, the risk for type 2 diabetes, and other complications of being overweight.

Implications of Early Onset

The longer a person has diabetes, the greater the chances he or she will sustain serious damage to the eyes, nerves, heart, kidneys, and blood vessels. This aspect of diabetes makes the growing burden of type 2 diabetes in children particularly alarming, as children with this diagnosis have a greater statistical chance of developing medical complications during their lifetimes. Primary prevention of type 2 diabetes in youth is therefore a key public health goal. However, optimizing type 2 diabetes treatment options is equally critical, in order to forestall the onset of complications in children who already have the disease.

The TODAY Trial

Therapeutic strategies for type 2 diabetes need to address the primary metabolic abnormality of this disease, which also underlies many of its complications—the inability to maintain blood sugar levels within a normal range. Many drugs are available to treat type 2 diabetes in adults, but metformin, which lowers the liver's production of glucose, is the only oral drug approved by the Food and Drug Administration to treat type 2 diabetes in children. The TODAY trial is examining the use of both metformin and another oral drug currently approved for adults only, rosiglitazone. Rosiglitazone belongs to a class of insulin-sensitizing drugs called the thiazolidinediones (TZDs). It helps muscle cells respond to insulin and use glucose more efficiently.

Enrollment in the TODAY trial began in Spring 2004 and is expected to continue for 3 years. TODAY participants will be randomly assigned to one of three treatment groups: metformin alone; metformin and rosiglitazone in combination; and metformin plus intensive lifestyle

change aimed at improving nutrition and increasing physical activity, with a goal of losing weight. Researchers plan to enroll 750 children and teens 10 to 17 years old diagnosed with type 2 diabetes in the past 2 years. The trial is expected to last 5 years.

The TODAY study's main goal is to determine how well and for how long each treatment approach controls blood glucose levels. The study will also evaluate:

- Safety of the treatments;
- Effects of the treatments on the following: insulin production; insulin resistance (a term denoting when cells do not effectively use insulin); body composition; nutrition; physical activity and aerobic fitness; risk factors for eye, kidney, nerve, and heart disease; quality of life; and psychological outcomes;
- Influence of individual and family behaviors on treatment response; and
- Cost-effectiveness of the treatments.

TODAY is the first clinical study to look at the effects of intensive lifestyle change aimed at lowering weight by cutting calories and increasing physical activity in youths with type 2 diabetes. The NIDDK-sponsored Look AHEAD trial is currently studying the benefits of weight loss in adults with type 2 diabetes. The TODAY trial is one of two NIDDK-funded studies that will focus on type 2 diabetes in children. An anticipated prevention study, currently in its pilot phase, will seek to develop cost-effective interventions that can be widely applied in schools across the country.

In a March 15, 2004 press release announcing the start of the TODAY study, then-HHS Secretary Tommy G. Thompson noted that: "Obesity and type 2 diabetes are among the most serious health challenges facing America's youth today. We need to do all we can to develop strategies that encourage healthy eating and active lifestyles in our children." By supporting major research studies aimed at the twin goals of optimal treatment and prevention of type 2 diabetes in children, the NIDDK hopes to ameliorate and possibly reverse the onset of this disease and its complications in this most vulnerable population.

TODAY Snapshot:
Participant Bethannie Ramirez


Bethannie Ramirez

Bethannie Ramirez is bright, mature, and articulate—and at the age of 13 was diagnosed with type 2 diabetes. Now 15 and a freshman in high school, Bethannie says that her diagnosis came as a real shock. "I thought, 'this couldn't happen to me!' But then I gradually started to accept it and said to myself, I might as well deal with it the best I can." Bethannie, who sports a 3.9 grade-point-average and is an avid reader—especially of the "Harry Potter" series—aspires to be a screen writer or perhaps even an actress one day. During one of her follow-up medical examinations, she was asked if she would be interested in taking part in the TODAY clinical trial. "I decided I would, not only for myself, but to help other kids my age better understand this disease and what they can do to help themselves," she says. With her parents' consent, she enrolled in the TODAY study in November 2004.

Bethannie, who is of Philippine decent and whose grandmother and uncle—both on her mother's side—have been diagnosed with type 2 diabetes, says that being part of the study is fitting into her life. "I take my medication, watch what I eat, and otherwise live normally," she says. She is also quick to add that she's happy with losing weight as a result of her participation in the study and that her high school friends are very supportive. "When I first was diagnosed, I didn't want to tell anyone," says Bethannie. "I told one person; then others found out. But no one pities me. They understand what I need to do [for my health] and respect me for doing it."

Bethannie's mother, Elizabeth, says she's very happy she enrolled Bethannie in the study and very proud that her daughter is volunteering to help other teenagers with type 2 diabetes. "She loves to volunteer for things," Elizabeth says. As for Bethannie, who likes to write poems and stories, she says that it is highly likely that one day she will write about her diabetes—and maybe even about taking part in the TODAY study.

National Diabetes Education Program (NDEP)

Through education and awareness campaigns and other health information dissemination efforts, the NDEP aims to improve treatment and outcomes for people with diabetes, to promote early diagnosis, and to help prevent the onset of diabetes. The program develops information and education messages and materials for people with diabetes and their families, health care providers, payers and purchasers of health care, health care system policymakers, and the general public—including people with undiagnosed diabetes and those at risk for the disease. The NDEP is jointly sponsored by the NIDDK and the Division of Diabetes Translation of the Centers for Disease Control and Prevention, both of the U.S. Department of Health and Human Services, and also involves the participation of over 200 public and private partner organizations.

The NDEP's "Small Steps. Big Rewards. Prevent Type 2 Diabetes" campaign is based on the findings of the NIH-sponsored Diabetes Prevention Program (DPP) clinical trial. The DPP demonstrated that the risk of developing type 2 diabetes can be significantly reduced through modest weight loss, of 5 to 7 percent of body weight, and exercise, such as 30 minutes of moderate physical activity five days per week. To reach those groups at high risk for type 2 diabetes, in 2004 the NDEP launched the first national multicultural diabetes prevention campaign, with tailored materials and messages for high-risk audiences. Campaign materials include motivational tip sheets, as well as print and radio public-service ads. For African-Americans, the NDEP's campaign is called, "More Than 50 Ways To Prevent Diabetes," and uses humor to encourage healthy lifestyle changes. For a Hispanic audience, the NDEP launched the campaign, "Prevengamos la Diabetes Tipo 2: Paso a Paso" (Let's Prevent Type 2 Diabetes: Step by Step). Campaign materials include a music CD, performed by Hispanic recording artists, to promote physical activity. For American Indians and Alaska Natives, the NDEP launched the public awareness campaign, "We Have the Power To Prevent Diabetes." The campaign uses testimonials from American Indians and Alaska Natives who have made lifestyle changes to prevent diabetes. For Asian Americans and Pacific Islanders, the NDEP's campaign, "Two Reasons I Find Time To Prevent Diabetes...My Future and Theirs," uses an inter-generational appeal to encourage people to make healthy lifestyle changes. The NDEP also is reaching out to older adults with the campaign, "It's Not Too Late To Prevent Diabetes. Take Your First Step Today." To help promote the campaign, NDEP has assembled a team of people from across the country who are working to prevent diabetes in their own lives and in their communities. Finally, for a general audience, the campaign has the message, "Get Real! You Don't Have To Knock Yourself Out To Prevent Diabetes." In 2005 the NDEP is adding a new target audience to promote diabetes prevention messages—women with a history of gestational diabetes and their children. Further information can be found at *http://ndep.nih.gov/campaigns/SmallSteps/SmallSteps_index.htm*.

In other activities, the NDEP continues to partner with the American Diabetes Association for the health awareness campaign, "Be Smart About Your Heart: Control the ABCs of Diabetes" to promote the link between diabetes and cardiovascular disease. Versions of the campaign are also tailored for Hispanic and Latino Americans and for Asian Americans and Pacific Islanders. The NDEP also offers other patient education materials and resources and tools designed for health care professionals, such as a new interdisciplinary primer for pharmacists, podiatrists, optometrists and dental professionals to promote a team care approach to comprehensive diabetes care with a companion supplement for controlling blood glucose, pressure and cholesterol. The NDEP web site (*betterdiabetescare.nih.gov*) provides information on making changes in systems of care that can lead to better delivery of care for people with diabetes. In 2004 NDEP revised the

publication, “Guiding Principles of Diabetes Care” outlining the seven essential components of quality diabetes care. The NDEP continues to provide the publication, “Helping the Student with Diabetes Succeed: A Guide for School Personnel,” and has developed a series of tip sheets for children with type 2 diabetes. For the worksite, the NDEP has a Web-based resource for employers and others; Spanish lesson plans were launched on the Website this past year to meet the growing need for Hispanic/Latino materials in the business community. The NDEP is beginning an initiative to determine the economic impetus for diabetes prevention and control. Further information on NDEP activities and materials can be found on the NDEP Website at: *http://ndep.nih.gov/index.htm*.

Enzyme Replacement Therapy for Lysosomal Storage Disorders

The body's cells recycle many of the substances they no longer need by digesting them with enzymes inside cellular compartments called lysosomes. If these enzymes are missing or defective due to genetic mutations, toxic waste products are not properly degraded. Instead, they build up in the lysosomes and lead to severe organ damage. Diseases caused by these enzyme deficiencies, referred to as lysosomal storage disorders, are individually rare, but collectively affect about 1 in 7,700 infants born in the United States.[1] Symptoms vary, and are often not apparent at birth; however, as the undigested materials accumulate, they can cause serious problems such as weakness, severe pain, brittle bones, mental retardation, corneal clouding, organ failure and death.

Lysosomal storage disorder research, built on substantial NIH investments followed by recent commercial product development, is a classic story of translating remarkable findings from basic research into Food and Drug Administration-approved treatments for three of these serious disorders: mucopolysaccharidosis I (MPS I), Gaucher disease and Fabry disease. The critical discovery dates to work in the late 1960s, when NIDDK intramural researchers found that growth medium taken from a culture of normal cells relieved the lysosomal storage defect of cells cultured from a patient with MPS I. In essence, this meant that normal cells secrete the enzyme missing in MPS I patients; and more importantly, the MPS I cells can internalize that enzyme from the medium, and somehow send it to the lysosome, right where it needs to go. It was later discovered that this secretion and re-uptake process is a pathway common to many of the enzymes absent in these disorders. Thus, in theory, patients with such a disease might be treatable by administering purified forms of the enzymes they need—an approach referred to as enzyme replacement therapy.

Indeed, experiments in the 1970s suggested that an enzyme-replacement approach could be beneficial. For example, in separate but related work, NIH intramural scientists and NIDDK grantees treated Gaucher and Fabry patients with the enzymes they lacked, which the researchers had purified from human tissue. These studies were of short duration, and the long-term health effects could not be determined. However, the accumulation of undigested lysosomal materials was significantly reduced for a period of time after treatment, at least in some parts of the body. Therefore, researchers theorized that, if adequate supplies of the enzymes could be produced, there was reasonable hope that they might be effective therapeutically.

Tremendous advances in gene manipulation technology in the 1980s made it possible to isolate the normal versions of genes mutated in patients with lysosomal storage disorders. Researchers showed that active, properly modified, human lysosomal enzymes could be produced in cultured mammalian cells. With this technology, comparatively large amounts of the enzymes could be produced and purified—far more inexpensively and easily than was previously possible.

However, there was a pressing need for an animal model of a lysosomal storage disorder to facilitate studies of the long-term safety and efficacy of such treatments. Therefore, another key finding was that a natural mutation occurring in some breeds of dogs eliminates the same enzyme that is missing in MPS I

patients. Because these dogs have symptoms quite similar to those of humans with the disease, they are a useful animal model that enabled pilot-tests of therapeutic strategies.

The combination of an improved enzyme supply and an animal model permitted testing of intravenous enzyme replacement therapy in MPS I dogs over a three month period. Some dogs developed immune reactions against the enzyme, but the problem could be managed through pre-medication with antihistamines and slower administration of the enzyme. More importantly, although lysosomal function remained unimproved in some parts of the body, including the brain, it was normalized in certain organs and greatly improved in others. With further work, methods were developed that avoided immune reactions from the animals, and enabled long-term treatment studies, as a prelude to clinical trials.

These ground-breaking basic and pre-clinical research advances were ultimately translated into valuable therapeutics by drug companies. Largely as a result of this translational research, the Food and Drug Administration granted approval for treatment of Gaucher, Fabry and MPS I patients with genetically engineered forms of their respective missing enzymes. The National Organization for Rare Diseases recognized this achievement by presenting its 2004 Corporate Awards to two of the companies which brought these products to market by building upon the earlier NIH-funded discoveries. Treatments for several more lysosomal storage disorders are currently in phase III clinical trials, and are likely to come to market soon.

As remarkable as these advances are, and although the improvements in quality of life for lysosomal storage disorder patients are potentially very significant, these treatments are not cures. Patients may have to see their physicians weekly to receive lengthy infusions of the enzymes. Moreover, some disease manifestations are unlikely to be alleviated, such as the corneal clouding, bone disease and mental retardation that often occur in MPS I patients. Therefore, the NIDDK continues to encourage research on lysosomal storage disorders.

One promising area for developing treatments that might avoid some of these limitations is the discovery of small molecules that can stabilize defective enzymes in patients in whom they are not entirely absent. To explore opportunities in this field, the NIDDK sponsored a workshop on “Protein Misfolding and Misprocessing in Disease.” As part of its Roadmap Initiative, the NIH is establishing small molecule screening facilities, which could speed up the process of identifying new therapeutics for lysosomal storage disorders.

[1] Meikle PJ, Hopwood JJ, Clague AE, and Carey WF. Prevalence of lysosomal storage disorders. *JAMA* 281: 249-254, 1999.

Denise Dengel

Living with MPS I Has Turned Her World Upside Down

Denise Dengel was once an avid outdoorswoman who loved to compete in equestrian barrel racing, hike and camp, work out at the gym, and ride mountain bikes with her friends. She says she remembers what it was like to be healthy, to feel good, to be working and living life to the fullest. "I was a very active woman," says 40-year-old Denise, who was forced to give up what she refers to as her "turbocharged" life by the time she reached her early 30s because of a rare metabolic disease, called MPS I.

MPS is shorthand for a group of seven inherited metabolic diseases called mucopolysaccharidoses. Over time, these diseases wreak havoc on joints and organs, resulting in permanent damage that can affect an individual's appearance, physical abilities, organ and system functions, and, in most cases, mental development. Denise's disease, MPS I, can be mild, intermediate, or severe, with symptoms ranging from joint stiffness and slow progression of physical problems in its milder form, to serious cognitive and physical impairment early on, and death in childhood at its most severe.

Unlike many MPS I patients, Denise has a relatively mild form of the disease, called MPS I, Scheie syndrome (MPS I S). Nevertheless, in recent years, this painful, incurable and insidious disease has dramatically affected her life, and without the development of fully effective treatments, will more than likely continue to do so.

Since the mid-1990s, Denise has had several operations, including two open heart surgeries,

Denise Dengel

an operation on each hand to correct for carpal tunnel syndrome, and another to remove toxic deposits on her spinal cord. She experiences headaches that she says feel "like a knife in my head." Her joints have stiffened to such a degree that she has had to give up most, if not all, of her physical activities, and her cognitive skills have decreased to the point that she has left her full-time job as a social worker and is now on long-term disability. Says Denise of her earlier, more healthy and active years: "I know what that world is like, and if I can't have it back, at the very least I don't want the world I currently live in to get any worse."

Hope Through Research

Research supported by the NIDDK has led to the development of the drug, Aldurazyme®, recently approved by the FDA as a treatment option for MPS I patients. (See also "Story of Discovery: Enzyme Replacement Therapy for Lysosomal Storage Disorders.")

PATIENT PROFILE

Denise began taking Aldurazyme® about a year and a half ago. She believes the drug has made her joints less stiff and thereby has greatly increased her flexibility. However, it is unlikely that Aldurazyme® will improve the cognitive and other brain-related symptoms Denise is experiencing because Aldurazyme® does not cross the blood-brain barrier—a special barrier system that protects the brain from absorbing harmful substances from blood. Thus, the NIDDK also is focusing its efforts on supporting research on methods, such as gene therapy, which may be able to prevent or treat brain damage in this disease. In the meantime, Denise says that, since taking Aldurazyme®, her joints "haven't gotten any worse," and adds that she is excited to see if this drug can at the very least stabilize her condition or better yet, improve it over time. "I get large doses of the enzyme I'm lacking. The hope is that the body takes up enough of it to retard or reverse the degenerative process that's taking place," she says.

About MPS

MPS is caused by the absence or malfunctioning of certain enzymes needed to break down glycosaminoglycans—long chains of sugar molecules used by cells to help build bone, cartilage, tendons, corneas, skin and connective tissue. (Glycosaminoglycans used to be called "mucopolysaccharides," hence the name of the disease.) Glycosaminoglycans, or GAGs, are also found in the fluid that lubricates joints. When these long sugar chains need to be broken down and recycled, cells haul them into special enzyme-filled compartments, called lysosomes, for digestion. People with MPS either do not produce enough of one of the 11 enzymes required to break down the GAGs into simple molecules, or they produce enzymes that do not work properly. In either case, the result is a toxic build-up of waste molecules in the body, as the lysosomes become engorged. Denise describes people with the disease as "being clogged everywhere with (GAGs), in our joints, as well as our organs."

There are many forms of MPS. MPS I S, Scheie syndrome, is the mildest form of MPS I. Symptoms, which include stiff joints, generally begin to appear after age five; a diagnosis is made most commonly after age 10. Children with MPS I S have normal intelligence or may have mild learning disabilities; some may have psychiatric problems. Glaucoma, retinal degeneration, and clouded corneas may significantly impair vision and eventually lead to blindness in adulthood. Other problems include carpal tunnel syndrome or other nerve compression, stiff joints, claw hands and deformed feet, a short neck, and aortic valve disease. Some affected individuals also have obstructive airway disease and sleep apnea. Unlike patients with many other forms of MPS, those with MPS I S can live into adulthood.

All forms of MPS, except MPS II, or Hunter Syndrome, are autosomal recessive disorders. This term means that only individuals inheriting the defective gene from both parents are affected. In Hunter Syndrome, the mother alone may pass the defective gene to a son.

Living with MPS I, Scheie Syndrome

Developmentally, Denise had a normal childhood, which even included horseback riding. But at some point her mother noticed that both Denise and her brother just didn't move like other children. "My brother's and my joints were beginning to stiffen from (GAG) build-up to the point where I couldn't get on my horse without assistance," says Denise. She and her older brother by 18 months were diagnosed with MPS I in 1975, when Denise was 10. Despite its genetic origins, there is no other known history of MPS in the Dengel family.

"Up until I was 25, I firmly believed that I had a disease that would only affect my joints. I thought that, as I got older, they would just continue to stiffen, and that nothing else was the matter with me."

At the time the siblings were diagnosed, not much was known about MPS. Both Dengel children were relatively mildly affected, and because neither was cognitively challenged by the disease, they were studied by researchers trying to find out more about this rare disease. Sadly, Denise's brother, with whom she was very close, died in an automobile accident at age 18.

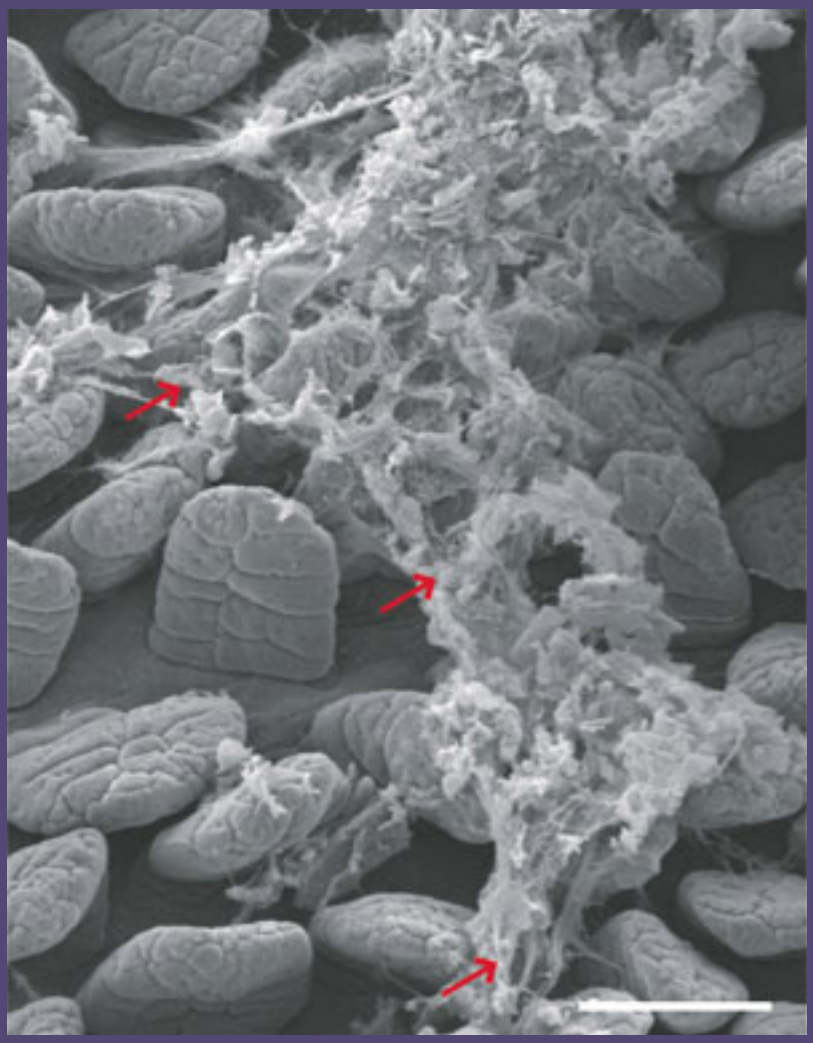
"Up until I was 25, I firmly believed that I had a disease that would only affect my joints. I thought that as I got older they would just continue to stiffen, and that nothing else was the matter with me," says Denise. Unfortunately, she was wrong. By her mid-20s, Denise began having problems with her hands, which led to carpal tunnel syndrome. Moreover, unbeknownst to her at the time, the disease was insidiously affecting other parts of her body as well, including her spine, heart, lungs and brain. "It has progressed to the point that today I'm a seriously affected adult with MPS I," says Denise.

By 1996, Denise was getting weaker and the disease was beginning to show up in her organs. For example, an MRI showed a build-up of GAG pressing on her spinal cord, so in 1997 she had spinal surgery near her brain stem. Unfortunately, because of its location, physicians could not remove all of the toxic material. In 1999, Denise had the aortic valve in her heart replaced with a tissue valve. Because of continued GAG build-up, the valve needed to be replaced again by 2002, this time with a synthetic component.

Denise says she suffers from neurological issues that include extreme headaches and dizziness. An MRI indicates that she also has lesions on the brain and is manifesting symptoms for hydrocephalus (water on the brain). "Sometimes I can't think clearly and my words become slurred. I often feel nauseated and suffer from chronic fatigue. If I'm lucky, I can function fairly well for two to four hours a day," she says. In 1998, because of these worsening conditions, Denise, who for 10 years gained great satisfaction from working with homeless youth in Seattle, Washington, quit her job as a social worker. But she's never given up on her life.

"I only know five people older than I am with my form of MPS who are still alive, and some of them are blind. I want nothing more than for research to change all of this within my lifetime."

"My family and friends are a great support system," says Denise. "I probably wouldn't be able to live as independently as I do without them." In 1996, Denise joined the MPS Society and served as a board member for two years. She says the society has become a "real mainstay" in her life. "We're a bunch of people trying to live the best that we can with what we've got," says Denise. She adds, "I only know five people older than I am with my form of MPS who are still alive, and some of them are blind. I want nothing more than for research to change all of this within my lifetime." She is currently a member of the MPS Society's federal legislative committee, which supports research efforts to help people with MPS. She's very excited about how much has been learned about MPS in recent years. "I'm a true believer in advocacy and education," says Denise.



Scanning electron micrograph of the small intestine of a mouse shows finger-like projections called villi underlying a layer of mucus. Villi on the inner surface of the intestine enable nutrients to be absorbed. The red arrows indicate portions of the gel-like layer of mucus that normally lines the intestine. The white scale bar at the bottom right, shown for reference, is 100µm long (1/10th of a millimeter, or about 1/250th of an inch). Image courtesy of Dr. Jeffrey Gordon and reprinted from Sonnenberg JL, Angenent LT, and Gordon JI. Getting a grip on things: how do communities of bacterial symbionts become established in our intestine?

Nature Immunol 5: 569-573, 2004.

Digestive Diseases and Nutrition

Digestive diseases are among the leading causes of hospitalization, surgery, and disability in the U.S. These conditions include disorders of the gastrointestinal tract, liver, gallbladder, and pancreas, as well as obesity and other nutrition-related disorders. Disorders of the digestive tract—such as irritable bowel syndrome and inflammatory bowel disease—exact a significant toll on many Americans each year. NIDDK-supported scientists are vigorously pursuing research to understand how widespread these diseases are across the U.S., to identify the causes of these diseases and how they progress, and to test new interventions for treatment and prevention of these costly diseases, including drugs, surgery, and behavior modification.

Several types of liver disease have serious adverse impacts on health, and some can lead to complete liver failure. Some liver diseases primarily affect children—such as biliary atresia, a progressive inflammatory liver disease—while others more commonly affect adults—such as non-alcoholic steatohepatitis (NASH). Some are caused by viral infection—such as hepatitis C—while others arise from diverse factors such as autoimmune reactions, genetic mutations, drug toxicity, and other, unknown triggers. A functioning liver is necessary for life, and the only treatment for end-stage liver disease is a liver transplant. The number of livers available from deceased donors is limited, and research is of critical importance to identify and treat liver disease, preserve liver function in people with liver disease, and explore treatment options beyond cadaveric liver transplants.

The number of overweight and obese Americans has risen dramatically in the past two decades and is now at epidemic levels. Obesity is associated with numerous serious diseases, including type 2 diabetes, heart disease, and cancer. While multiple factors contribute to obesity, caloric intake clearly plays a key role in weight gain. As scientists elucidate the molecular factors that control appetite, metabolism, and energy storage, they are identifying potential targets for the development of new pharmacologic agents to promote safe, long-term weight loss. Investigators are also continuing behavioral research to help people achieve healthy lifestyles that include increased physical activity and improved diet. (Additional information on NIDDK-supported research endeavors focusing on obesity is provided in the next chapter.)

Intestinal disorders include functional bowel disorders, which result in symptoms of abdominal pain and altered bowel habits. For example, irritable bowel syndrome (IBS) causes pain and constipation or diarrhea. IBS more frequently affects women, who may display a different range of symptoms and respond differently from men to pharmacologic treatments for the disease. While diet and stress contribute to this disorder, its underlying causes are unknown.

Inflammatory bowel disease (IBD), which includes Crohn's disease and ulcerative colitis, is marked by destructive inflammation in the intestinal tract leading to rectal bleeding, diarrhea, nutritional deficiencies, and other serious complications. To address this condition, surgery may be required, including removal of the affected region of the intestine. Scientists are dissecting the complex interactions among the genetic, environmental, and cellular factors that contribute to the development of IBD. Helping to catalyze the design of novel therapeutic strategies will be the continued identification of predisposing genetic variations and their interactions, as well as other factors, such as potential autoimmune and microbial influences.

The microorganisms that inhabit the gastrointestinal tract are powerful players in maintaining or tilting the balance between digestive health and disease. These microbes can affect intestinal health in some surprising ways, depending on their interactions with cells of their host. Scientists are gaining insights into the ways these microorganisms influence the development and function of the digestive tract.

Some digestive diseases can be triggered by the body's reaction to certain foods. In individuals with celiac disease, the small intestine is damaged when the immune system reacts to the protein gluten—a component of wheat, barley, and rye. This reaction interferes with the ability to absorb nutrients from foods and can result in chronic diarrhea, bloating, anemia, and, in children, growth failure. The only current treatment for celiac disease is maintenance of a gluten-free diet, which is difficult for many people. The greater challenge now facing patients and their healthcare providers is to improve methods capable of diagnosing celiac disease early, before damage occurs or other conditions develop. Recent and continued advances in the understanding of genes that predispose individuals to develop celiac disease may contribute to improved diagnosis in the future through genetic-based screening.

BOLSTERING LIVER DISEASE RESEARCH AT THE NIDDK

Action Plan for Liver Disease Research: Liver disease is an important cause of morbidity and mortality in the United States, affecting persons of all ages, but most frequently individuals in the productive years of life, between the ages of 40 and 60 years. Liver disease also disproportionately affects minority individuals and the economically disadvantaged. Medical research on liver disease is critically important and further progress in research promises to reduce the major toll of liver disease on human health and well-being. Indeed, the last 25 years of medical research in liver disease has resulted in major improvements in the survival and quality-of-life of patients with liver disease. The future should bring even more profound changes.

To address the burden of liver diseases in the United States, a subcommittee of the Digestive Diseases Interagency Coordinating Committee, led by the NIDDK, has developed an Action Plan for Liver Disease Research. The Action Plan addresses the broad range of liver disease research, and is organized around 16 topic areas. The overall goal of the Action Plan for Liver Disease Research is to advance research on liver disease with the aim of decreasing its burden in the U.S. For more information about the trans-NIH Action Plan for Liver Disease Research, see page 46.

Immune Cell Transplantation for Liver Disease of Hereditary Tyrosinemia Type I: New approaches for treating liver disease are emerging from laboratory studies of hereditary tyrosinemia type I—an inherited metabolic disorder associated with severe liver disease in infants and children. It is caused by a deficiency in an enzyme that breaks down the amino acid tyrosine, resulting in elevated tyrosine levels in the blood (tyrosinemia) and tissue damage. A drug for treating this disease was approved in 2002 which provides a means of long-term control of tyrosinemia. In cases of advanced disease, however, liver transplantation is the only effective current therapy. One way to correct the underlying defect in genetic diseases such as hereditary tyrosinemia would be through transplantation of cells with a functioning copy of the gene for the missing enzyme. In recent years, researchers have explored this possibility in mice that are deficient in the same enzyme that causes hereditary tyrosinemia type I in humans. They found that transplantation of stem cells derived from the bone marrow of healthy adult donor mice into the mice with tyrosinemia resulted in fusion between the healthy and diseased cells, correction of the genetic defect, and repair of the liver. However, the question remained of whether stem cells, with their ability to turn into a variety of cell types, were required, or if more mature cells—already committed to forming a particular cell type—could also work to correct the defect. Researchers addressed this by conducting a series of transplantation experiments using several different types of donor mice that were genetically engineered to produce only certain types of cells that originate in the bone marrow. They found

As a result of new insights into the understanding of the underlying causes of celiac disease, and in order to improve awareness, diagnosis, and management of this condition, in June 2004 the NIDDK and several other Institutes and Centers of the NIH convened a Consensus Development Conference on Celiac Disease. This conference examined the current state of knowledge and identified directions for future research. The 13-member panel included practitioners and researchers in gastroenterology, pediatrics, pathology, internal medicine, endocrinology, a dietitian, a geneticist, and a consumer representative. The panel made recommendations for future efforts to study and treat celiac disease. Illustration courtesy of the NIH Office of Medical Applications of Research, which co-sponsored the meeting and commissioned the image as part of the Conference.

that it was possible to correct the defect in mice with tyrosinemia by using macrophages—a kind of immune cell that develops from cells that form in the bone marrow. These results support the theory that donor stem cells used in prior experiments probably differentiated into macrophages prior to fusing with the recipient's liver cells. Importantly for potential clinical applications, this study also suggests that, in contrast to bone marrow transplantation, treatment with macrophages could be a less invasive, more efficient type of cell transplantation procedure for genetic liver diseases, such as hereditary tyrosinemia. A key benefit of macrophages or their immediate precursor cells is that they could be administered directly into the liver or bloodstream.

Willenbring H, Bailey AS, Foster M, Akkari Y, Dorrell C, Olson S, Finegold M, Fleming WH, and Grompe M. Myelomonocytic cells are sufficient for therapeutic cell fusion in liver. *Nat Med* 10: 744-748, 2004.

While research aimed at determining ways to allow patients with end-stage liver disease to regenerate liver tissue holds hope for the future, researchers are seeking ways to expand the pool of organs available for transplant. Presently, more than 5,000 liver transplants are performed every year. Unfortunately, more than 17,000 patients are awaiting liver transplantation, and in recent years, the waiting list has continued to grow. As a consequence, the numbers of patients dying while on the liver transplant waiting list has grown.

Because of the shortage of donor livers available from cadavers, transplants from living donors have been the subject of much interest. While living donor liver transplantation has become widely accepted in pediatric patients, its use in adults is controversial, as the procedure is challenging and potentially dangerous. Between 1998 and 2003, at least two healthy, adult donors died after adult-to-adult living donor liver transplantation surgery.

To address the issues of the proper use, relative risks, and potential benefits of adult-to-adult living donor liver transplantation, the NIDDK established a multi-center clinical study. The "Adult-to-Adult Living Donor Liver Transplantation Cohort Study" (A2ALL) consists of nine liver transplant centers experienced in performing living donor liver transplantation and a data coordinating center responsible for maintaining the database on patients. The primary goal of A2ALL will be to provide valuable information on the outcomes of living donor liver transplantation. This important study will follow both donors and recipients before and after the liver transplant operation, assessing clinical outcomes and quality of life. This information will help aid decisions made by physicians, patients, and potential donors.

Combination Drug Therapy Effective for Hepatitis C – Research Advance from the NIDDK's HALT-C Clinical Trial: The hepatitis C virus (HCV) is the most common cause of liver disease in the US. About four million Americans have been infected with HCV, and most of them now have chronic hepatitis. In some, chronic hepatitis C leads to cirrhosis, liver failure, and liver cancer. Liver failure due to chronic hepatitis C is the most common cause of liver transplants. The only treatment proven to be effective for hepatitis C is interferon, with or without the antiviral drug ribavirin. Unfortunately, many patients treated with these medications do not respond, and the virus continues to cause liver damage. The NIDDK is supporting studies to determine if continuing interferon long-term in patients who remain infected with HCV may prevent progressive liver damage. The "Hepatitis C Antiviral Long-Term Treatment against Cirrhosis" (HALT-C) clinical trial is designed to determine if long-term treatment with pegylated interferon—a form of interferon chemically modified to make it longer-acting—in people with HCV who have not responded to previous interferon-based therapy can prevent cirrhosis and reduce the risk of developing end-stage liver disease and liver cancer. Patients enrolled in HALT-C initially receive a 24-week course of a combination of pegylated interferon and ribavirin, an antiviral drug. Patients who respond to this combination therapy continue for another 24 weeks; those who do not respond are randomized either to continue to receive pegylated interferon or to stop treatment. Patients will be followed for up to four years.

HALT-C researchers have recently reported the results of an early portion of the study. They asked whether patients with HCV who had previously been treated with—and not responded to—unmodified interferon alone would respond favorably to the combination therapy. Over 600 patients who were nonresponders to previous interferon therapy, with or without ribavirin, were treated with pegylated interferon plus ribavirin. After 20 weeks, 35 percent of the patients had no detectable evidence of HCV infection in their blood. These patients continued on therapy for a total of 48 weeks. Following discontinuation of therapy, 18 percent of the initial number of patients achieved a sustained virologic response, which means that the virus remained undetectable in their blood. This study suggests that some patients who did not respond to initial therapy with interferon may benefit from re-treatment with pegylated interferon and ribavirin.

Shiffman ML, Di Bisceglie AM, Lindsay KL, Morishima C, Wright EC, Everson GT, Lok AS, Morgan TR, Bonkovsky HL, Lee WM, Dienstag JL, Ghany MG, Goodman ZD, and Everhart JE, and The HALT-C Trial Group. Peginterferon alfa-2a and ribavirin in patients with chronic hepatitis C who have failed prior treatment. *Gastroenterology* 126: 1015-1023, 2004.

The NIDDK is also pursuing multiple avenues of research into therapies for hepatitis C in diverse populations. It is known that African Americans with hepatitis C respond less well to interferon-based therapies, compared to Caucasians. In fact, several studies have found that the sustained response rate to interferon among African Americans is one-third to one-half of that seen in whites. To investigate possible reasons for this difference and identify possible improvements in treatment regimens, the NIDDK has funded the "Study of Viral Resistance to Antiviral Therapy of Chronic Hepatitis C" (Virahep-C). This is a clinical trial designed to investigate the best available medication for treating hepatitis C, to see how well these medications work in African Americans and Caucasian Americans, and to study the reasons that treatment for hepatitis C works for some patients, but not for others. Patients are being treated with a combination of pegylated interferon and ribavirin for one year. Patients are then followed for another year after treatment. The Virahep-C trial may provide important information about how well African Americans respond to treatment for hepatitis C compared to Caucasian Americans; factors that may predict response to

treatment, especially in African American patients; and how the patients' genes, especially those controlling the immune system, affect response to treatment. The trial is expected to be completed in 2006.

The NIDDK is also concerned about the special problems of children infected with the hepatitis C virus. In the recently-launched clinical trial "Peginterferon and Ribavirin for Pediatric Patients with Chronic Hepatitis C" (Peds-C), approximately 120 children will be randomly assigned to receive either pegylated interferon alone or pegylated interferon and ribavirin for 48 weeks. The children are carefully monitored for evidence of liver disease and hepatitis C virus levels, as well as for any side effects of therapy, their growth and development, and quality of life. A long-term follow up study of the clinical trial participants is planned. This study is also receiving support from the Food and Drug Administration, as well as from industry.

Drug-induced Liver Injury: Every year, many people inadvertently suffer severe liver injury from prescription and "over-the-counter" medications, nutritional supplements, alternative medicines, and herbal preparations. Most drugs are safe for the majority of patients taking them, and the reason that some patients are susceptible to liver injury from a drug is rarely known. Drug-induced liver injury occurs in all age groups, but most cases are seen in older people, because they take more medications than younger persons and also use multiple medications. Their ability to metabolize drugs in the liver may also be less than that of younger people. Unfortunately, the extent and magnitude of the problem are not well understood, because definitions and data for drug-induced liver injury are suboptimal. To address this gap in knowledge, the NIDDK has launched a "Drug-induced Liver Injury Network" (DILIN). One objective of the Network is to develop standardized definitions and tools to identify and fully characterize cases of drug-induced liver injury. With systematic classification, researchers will be better able to analyze drug-induced liver injury and collect biological samples from patients that can then be used to study the causes of liver toxicity. Another objective of the Network is to establish a registry of patients who have experienced severe drug-induced liver injury. The Network should enable researchers to develop better ways to prevent, detect, and treat this growing liver problem.

CELIAC DISEASE

Children at Risk for Celiac Disease May Have Subclinical Symptoms: People with celiac disease develop severe digestive problems when they eat gluten, a major protein component of grains such as wheat, rye and barley. A small amount of any of these foods is all that is required to damage the intestines of susceptible individuals, limiting the absorption of vital nutrients. Malabsorption slows physical development in children, and can cause a host of other symptoms. Definitive testing for celiac disease requires an intestinal biopsy, and the disease often goes undiagnosed. However, a blood test can identify at-risk individuals. Thorough screening of children born in Denver, Colorado, between 1993 and 1999 has shown that about 0.9 percent of children develop the disease by age five. A new study compares 18 children found to be at-risk, but who had not developed overt celiac disease, to 100 age- and gender-matched controls. The at-risk children had higher rates of some celiac disease symptoms, including irritability/lethargy and abdominal distension/gas, and were found to grow more slowly than their peers. These differences were small but statistically significant.

Hoffenberg EJ, Emery LM, Barriga KJ, Bao F, Taylor J, Eisenbarth GS, Haas JE, Sokol RJ, Taki I, Norris JM, and Rewers M. Clinical features of children with screening-identified evidence of celiac disease. *Pediatrics* 113: 1254-1259, 2004.

Hoffenberg EJ, MacKenzie T, Barriga KJ, Eisenbarth GS, Bao F, Haas JE, Erlich H, Bugawan TL, Sokol RJ, Taki I, Norris JM, and Rewers M. A prospective study of the incidence of childhood celiac disease. *J Pediatr* 143: 308-314, 2003.

Consensus Development Conference: In June 2004 the NIH convened a Consensus Development Conference on celiac disease. The 13-member panel included practitioners and researchers in gastroenterology, pediatrics, pathology, internal medicine, endocrinology, a dietitian, a geneticist, and a consumer representative. The panel reviewed an extensive collection of medical literature related to celiac disease. The panel concluded that celiac disease is under-diagnosed, and recommended increasing physician awareness of its various manifestations and appropriate use of available testing strategies. These proposed changes may lead to earlier diagnosis and better outcomes for patients. Based on its assessment of an extensive collection of medical literature and expert presentations, the panel identified six elements essential to treating celiac disease once it is diagnosed: (1) consultation with a skilled dietitian, (2) education about the disease, (3) lifelong adherence to a gluten-free diet, (4) identification and treatment of nutritional deficiencies, (5) access to an advocacy group, and (6) continuous long-term follow-up.

To address the recommendations of the Consensus Conference, the NIDDK is planning in early 2005 to discuss the development of a celiac disease awareness program with stakeholders—including patient advocates. The objective of such a program would be to increase the likelihood that primary care providers would recognize and take appropriate diagnostic steps for patients who might have celiac disease. Such an effort would address the Consensus Conference's finding that celiac disease is largely undiagnosed in the U.S., possibly because initial recognition of the disease is most likely to occur in the primary care setting.

BACTERIA IN THE GUT

Although many strains of bacteria cause illness, not all bacteria are unwanted; "good" bacteria live throughout the body with benefit to both host and microbe. When one thinks of bacteria, disease-causing microbes may be the first to spring to mind, such as the ones responsible for urinary tract infections, for example. However, the environment is full of bacteria that are benign or beneficial. Indeed, it has been estimated that between 500 and 1,000 different species of bacteria inhabit the human digestive tract. While the relationship between the body and disease-causing bacteria is relatively well understood, less is known about how the body and its associated "good" bacteria influence each other. In some conditions, for unknown reasons, the body mounts an immune response against "good" bacteria, triggering an inappropriate reaction that may contribute to conditions such as Crohn's disease. NIDDK-supported scientists are working to better understand the relationship between both good and bad bacteria, and the body's immune system, and protection against or vulnerability to disease.

Symbiotic Bacteria May Promote Intestinal Health: The remarkably complex microenvironment of the intestine contains an abundance of microorganisms that provide health benefits to the host by inducing tolerance to substances in the environment or food that trigger an immune response. Although the gut is the site of rapid turnover of cells and propulsion of food and water, some microbes are able to latch-on to and colonize the intestine. Scientists continue to search for factors that differentiate microbial "residents" (those that successfully colonize)

versus "tourists" (those that merely pass through the digestive system). The factors of interest are those that can promote initial gut attachment and resistance to the wash-out of beneficial bacteria, as well as factors that inhibit colonization of harmful ones. Recent studies suggest that the polysaccharide-rich mucus gel layer of the human intestinal wall provides a matrix capable of supporting a thin layer of helpful bacteria that functions to aid digestion of intestinal contents and augment host defenses against disease causing organisms. Events leading to the interruption of this symbiotic relationship may promote an immune response to specific microbes. Emerging data indicates that *B. thetaiotaomicron*, a predominant member of the intestinal bacteria, induces intestinal Paneth cells to secrete a protein called angiogenin-4. Angiogenin-4 kills certain types of bacteria and may function to prevent microorganisms from invading the intestinal lining. Hence, specific bacteria of the gut may regulate expression of natural antibiotics and regulate the microbial ecology of the intestine. These findings may lead to the development of therapeutic and preventative strategies to support beneficial bacteria or impede the effects of those that cause disease.

Sonnenburg JL, Angenent LT, and Gordon JI. Getting a grip on things: how do communities of bacterial symbionts become established in our intestine? *Nat Immunol* 5: 569-573, 2004.

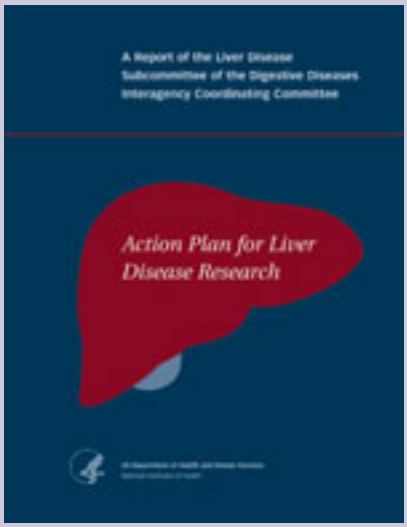
A Gene Expressed in Paneth Cells May Contribute to Crohn's Disease: Crohn's disease is a chronic, currently incurable digestive disease, most commonly affecting either the colon or the portion of the small intestine nearest to it, the ileum. Symptoms frequently include abdominal pain, nausea, vomiting, weight loss, and diarrhea, which is occasionally bloody. The precise causes of Crohn's disease are unknown, but bacteria in the gut are thought to contribute. There may also be a genetic component: the disease not only runs in families, but Americans and Europeans with the disease also frequently have particular variants of a gene called *card15,* which is expressed in immune cells, and believed to have a role in innate immunity to bacteria. In a new study, researchers found that the *card15* gene is expressed at high levels in so-called Paneth cells, which lie at the base of invaginations in the small intestine. The Paneth cells secrete anti-microbial compounds, probably playing an important role in controlling gut bacteria. Thus, *card15* and Paneth cells represent an apparent link between the genetic and bacterial risk factors for the illness, and are a promising target for development of therapeutics.

Ogura Y, Lala S, Xin W, Smith E, Dowds TA, Chen FF, Zimmermann E, Tretiakova M, Cho JH, Hart J, Greenson JK, Keshav S, and Nuñez G. Expression of NOD2 in Paneth cells: a possible link to Crohn's ileitis. *Gut* 52: 1591-1597, 2003.

Advancing Liver Disease Research Across the NIH: The Action Plan for Liver Disease Research

Liver and biliary diseases affect Americans of all ages and walks of life. An estimated 5.5 million Americans currently have chronic liver disease or cirrhosis, while more than 20 million have gallbladder disease. Progress in controlling liver and biliary disease depends largely on advances in understanding of these diseases through biomedical research. An *Action Plan for Liver Disease Research* has been developed to respond to the need to advance research on liver and biliary diseases, with the ultimate aim of decreasing the burden of these diseases in the United States. The focus of the trans-NIH Action Plan is on identifying areas of scientific opportunity leading to practical but important goals in the prevention and control of liver and biliary diseases that could be pursued with NIH support over the next decade. The *Action Plan* addresses the broad range of liver disease research, and is organized around 16 topic areas:

- Cell and Molecular Biology of the Liver;
- Liver Injury, Inflammation, Repair, and Fibrosis;
- Developmental Biology and Regeneration;
- Bile, Bilirubin, and Cholestasis;
- Viral Hepatitis;
- HIV and Liver Disease;
- Fatty Liver Disease;
- Drug- and Toxicant-Induced Liver Disease;
- Autoimmune Liver Disease;
- Pediatric Liver Disease;
- Genetic Liver Disease;
- Liver Transplantation;
- Complications of Liver Disease;
- Liver Cancer;



The *Action Plan for Liver Disease Research* aims to advance NIH-supported research on liver diseases with the ultimate goal of decreasing their burden in the United States. It describes recent research advances and identifies important research goals to pursue over the next decade. The *Action Plan* was developed with broad external input from the research, professional, and patient-advocacy communities. Its development was directed by the Liver Disease Subcommittee of the statutory Digestive Diseases Interagency Coordinating Committee.

- Gallbladder and Biliary Disease; and
- Liver Imaging and Biotechnology.

The *Action Plan for Liver Disease Research* was developed in response to congressional interest in bringing additional focus to liver research supported by the NIH and in response to the public health burden of digestive diseases and emerging research opportunities to address them. The NIH made a commitment to building on the robust liver research portfolio in order to bring greater focus and coordination to liver disease research

supported by the NIH. A special focus on liver disease research was initiated by NIDDK in July 2003, when the Institute's Director established the Liver Disease Research Branch within the Division of Digestive Diseases and Nutrition (DDDN) of the NIDDK. The NIDDK Director appointed an internationally-recognized authority in liver disease to lead this Branch, which pursued the formation of a Liver Disease Subcommittee within the statutory Digestive Diseases Interagency Coordinating Committee (DDICC). This committee is a group of representatives from across the NIH and other Federal agencies that serves to coordinate research efforts combating digestive diseases.

The Action Plan represents the broad input of a diverse and talented group of individuals who are committed to advancing liver disease research, including those from the NIH and other Federal agencies, as well as intramural and extramural researchers, physicians, and representatives of professional and patient advocacy groups. This broad input was gained through several modes, including an open meeting, Working Groups and Primary Review Groups, and an invitation for public comments on the draft Action Plan through the Internet. The Action Plan can be accessed in electronic form through its website: *http://liverplan.niddk.nih.gov*. Hard copies of the publication are also available, and ordering information is provided on the website. The plan also outlines ten research goals, or "benchmarks," to gauge overall progress in advancing liver disease research. The benchmarks are to:

- Improve the success rate of therapy of hepatitis C;
- Develop effective therapies that can be used in fatty liver disease, both alcoholic and nonalcoholic;
- Develop regimens of antiviral therapy that are effective in the long-term management of hepatitis B;
- Develop sensitive, specific, and noninvasive means of assessing disease stage (i.e., extent of fibrosis) in chronic liver disease;
- Develop sensitive and specific means of screening individuals at high risk for early hepatocellular carcinoma;
- Develop means to prevent gallstones;
- Elucidate the cause of biliary atresia;
- Improve the safety and define optimal use of living donor liver transplantation;
- Develop standardized and objective diagnostic criteria of major liver diseases and their grading and staging; and
- Decrease the mortality rate from liver disease.

PATIENT PROFILE

Allen Russell

Liver Transplant for Alpha-1 Antitrypsin Deficiency Affords a New Lease on Life

Allen Russell's life was pretty much free of any serious illnesses. However, about the time he reached his mid-40s, he started experiencing shortness of breath. An allergist said it was related to asthma and started Allen on therapeutic inhalants. Allen, a smoker since his college days, saw a correlation and decided that it was time to quit. However, despite using the inhalants and quitting cigarettes, Allen's condition did not appear to improve.

In the spring of 1999, in follow-up to an earlier, unrelated blood test that had indicated slightly abnormal liver enzyme levels, Allen had a liver biopsy. Test results from the biopsy came back positive for alpha-1 antitrypsin deficiency (AAT deficiency, also called Alpha-1), one of the most prevalent, potentially lethal hereditary disorders. AAT deficiency can cause life-threatening lung disease and/or liver disease in adults and children.

"My gastroenterologist said that, in his more than 20 years of practice, he had never seen a case of alpha-1 antitrypsin deficiency before this," says Allen. But the physician did encourage Allen to see a hepatologist. This specialist later informed Allen that, given the results of his biopsy and several other tests, he would most likely need a liver transplant in 3 to 10 years. The diagnosis took Allen entirely by surprise. A former athlete, he always felt that he was the picture of health. In September 2002, just 3 years and 4 months after his diagnosis, Allen's life was saved by a liver transplant.

Today, Allen is doing well with his new liver and leading a very active life, much of it devoted to promoting organ donations and helping others get through

Photo credit – Rick Brady

Allen Russell

serious health problems. He is very grateful to the anonymous donor whose liver he received and to the donor's family. "Up until my transplant I thought my faith was strong," says Allen. "The transplant only bolstered my faith and my need to help others." But it was a long and scary journey for Allen and his family before getting to that point.

About Alpha-1 Antitrypsin Deficiency

Alpha-1-antitrypsin, or AAT, is a protein produced mostly by the liver. Its primary function is to protect the lungs from an enzyme, neutrophil elastase, that normally digests damaged or aging cells and bacteria in order to keep the body healthy. If there is insufficient AAT circulating in the bloodstream, the destructive action of the damaging enzyme is left largely unchecked, leading to the destruction of healthy lung tissue and resulting in conditions such as emphysema, chronic bronchitis and lung infections.

However, while too little AAT is responsible for lung damage in this disease, "too much" AAT may be responsible for damage to the liver. In the most common form of symptomatic AAT deficiency, an alteration in the AAT protein that inhibits its secretion from cells in the liver—and hence, reduces AAT levels in the bloodstream—also leads to its abnormal accumulation within liver cells. It appears that this buildup of AAT induces cellular responses that can eventually injure the liver cells and cause overall liver damage, such as cirrhosis. Because liver damage only occurs in a subset of AAT deficient patients, it is thought that other factors, environmental and/or genetic, also influence how the liver cells handle the buildup of AAT and thereby play a role in determining whether a patient progresses to liver disease. Thus, the same inherent flaw in the AAT protein may inflict damage on two major organs—the lungs and liver—by entirely different but intimately linked mechanisms. (See also "Story of Discovery: Flaws in Protein Processing: Insights from Alpha-1 Antitrypsin Deficiency.")

Although it can be diagnosed in adulthood—Allen was diagnosed at age 46—AAT deficiency is an inherited disease and the most common genetic cause of liver disease in children. It most often appears in the newborn period with jaundice, swelling of the abdomen, and rejection of food.

According to the Alpha-1 Foundation, a not-for-profit organization dedicated to finding a cure for AAT deficiency, it is estimated that 20 million Americans are undetected carriers of the disease-causing gene and may be at risk for lung or liver disease; 100,000 individuals are actively lung- or liver-affected with fewer then ten percent (10,000) accurately diagnosed. Fortunately, for reasons still not understood, only 10 to 20 percent of those diagnosed with the deficiency will progress to liver disease. Although certain abnormalities associated with the liver disease can be treated or controlled, primarily with vitamin supplements, there is no cure for AAT deficiency.

For those patients who do suffer progressive liver damage to end-stage liver disease, the only option for survival, as Allen learned, is a liver transplant.

Living with AAT Deficiency

Allen was born with a severe case of jaundice and had to be re-hospitalized for 3 to 4 days only 6 weeks after his birth. "I didn't have much of an appetite, and what I did eat, I'd throw up instantly," he says. "The doctors didn't know if my bile ducts were functioning or not. The ducts eventually began to function normally." The acute problems soon passed, and because there is no known history of AAT deficiency in Allen's family, the incident was never linked to the disease. In fact, AAT deficiency was not even named until the late 1960s, about 14 years later.

For decades, Allen never gave a second thought to his neonatal bout with his jaundice and proceeded to lead an active, productive life. In addition to swimming, playing baseball, golf and tennis from boyhood to high school, Allen says he played scout team quarterback for the Georgia Tech Freshman Football Team, and was the second fastest miler on that squad. "I had no clue anything was wrong with either my lungs or my liver," he says. After college, Allen married, had two sons, and started out on a successful professional career in management with South Central Bell in Birmingham, Alabama.

However, in 1996, at age 43, Allen was told that his liver enzymes were a bit out of the normal range and that this condition should be monitored, but it didn't seem to Allen at the time to be very serious. In 1998, he began feeling shortness of breath and started on inhalants. Unbeknownst to Allen, AAT deficiency was affecting both his liver and lungs. A year later, he had the liver biopsy and was told he would need a liver transplant in 3 to 10 years. "I was stunned," says Allen. At first, he only told his wife and parents. A few weeks later he told his boss and later broke the news to his two teenage sons that he would eventually need a new liver.

PATIENT PROFILE

Coping with the Diagnosis

Since 10th grade, Allen had kept a journal. With this new knowledge of his AAT deficiency and the looming transplant, he began writing in it more frequently and earnestly. Eventually, he created a network of very close and trusted friends and relatives to whom he often turned "to talk through some new twist" in his journey, or "to share good or bad news." He also began researching AAT deficiency and liver transplants on the Internet, looking for whatever information he could find. "I even visited medical libraries," he says. "Learning as much as I could is how I dealt with my fear."

Allen's disease continued to progress. A second liver biopsy indicated that his cirrhosis was worsening. Three months later a blood test indicated that he had a severe shortage of platelets, the part of the blood necessary for clotting. By early 2002, Allen was experiencing bleeding in his esophagus and pains in his lower abdomen. By mid 2002, as a side effect of his declining liver, he began losing focus at work. By this time, he already was on the waiting list to receive a liver transplant. With accelerating emphysema and an aneurysm discovered near his liver, he moved up the waiting list.

Life-Saving Transplant

By nature, Allen is an organizer and planner. Just in case a liver from a deceased donor did not become available in time, Allen had three viable and willing living donors lined up, including his brother, a cousin-in-law and a very good friend. One of these candidates could have contributed a part of his or her liver to Allen, in a procedure called living-donor liver transplantation. But on September 5, 2002, at precisely 9:08 a.m., as Allen vividly recalls, he answered the phone to learn that a liver was available for him. His relief resulted in tears, he says, and within about 6 hours he arrived at the Vanderbilt University Medical Center, his carefully selected location for the transplant. He received his new liver early the next morning, within only a few blocks of his jaundiced birth about 49 years earlier.

"Dear Donor Family Member(s),

My name is Allen and I'm very fortunate to have received a liver from your family member.... The donation...has helped me survive and gives me hope for many more years of happiness with my family and friends. I am sincerely grateful for this gift, and truly appreciate any part you had in making this possible."

Allen's recovery from surgery was so remarkably fast and went so well that he was released from the hospital in a week, then from the nearby hotel tailored for patient rehabilitation just two weeks later. Having expected to remain in Nashville for at least 6 weeks, Allen was delighted! "That gave me a great bit of confidence," he says. By the end of October, however, his body began rejecting the new liver. Three days on heavy doses of steroids managed to stop the rejection. However, Allen experienced another rejection episode in November. This time, he was placed on different anti-rejection drugs and, according to Allen, "they seem to have done the trick. I've had no rejection of my liver since. My body just had to get compatible with the right drugs. I remain extremely grateful to my donor for my second chance in life." Although Allen's new liver is functioning well and has provided the needed AAT protein to his lungs so that further lung damage can be diminished, transplantation is not the ultimate cure for all AAT deficiency patients. Moreover, a number of factors, such as the limited supply of cadaveric livers available for transplantation, a lengthy waiting list, and cost, can ultimately prevent patients in need of a transplant from receiving this gift. Thus, developing better treatments for AAT deficiency will depend upon continued research on the underlying disease mechanisms, as well as continued careful assessment of new approaches to liver replacement, such as living donor liver transplantation.

Says Miriam O'Day, the Alpha-1 Foundation's senior director of public policy, "The organizations that advocate for individuals with Alpha-1 have tried to raise awareness that this is a liver disease that usually manifests clinically as a lung disease, making collaboration across disciplines essential." She adds that the public investment in research being conducted at NIDDK into liver disease, and AAT deficiency specifically, remains critical. In addition, the Foundation has invested significant funds from private donors into solving this problem.

By December 5, 2002, just three months after his transplant operation, Allen returned to his employer of over 20 years, BellSouth, to work half days, and a week later, returned to a full workload. "I was so excited to be back that my boss put me in charge of our group's morale!"

Today, Allen lives life at full tilt. He is a board member for the Alpha-1 Association, a member-based nonprofit organization founded in 1991 to identify those affected by AAT deficiency and to improve the quality of their lives through support, education, and advocacy. (The Alpha-1 Association is distinct from the Alpha-1 Foundation.)

The Association served to launch Allen's quest for knowledge about the disorder, and got him involved in a research project that helped him understand his illness and ways to cope. He has been a volunteer speaker and health fair resource for Georgia LifeLink, which promotes organ donations. He writes articles about his transplant for various publications, including one for the American Liver Foundation, and he maintains a website describing his transplant experience.

Allen serves on the board of the Kiwanis Club of Peachtree City, Georgia, and is a deacon for the First Presbyterian Church of Peachtree City. He is an active Georgia Tech alumnus. Allen's most recent project is "The Lighthouse Team," an on-line support group for patients, friends and families seeking advice and encouragement to help get them through the many stages of liver disease, transplantation, and on the road to wellness.

"My life's mission is to help people feel that there really is a light at the end of *their* tunnel," says Allen, who, now nearly 52 years old, is grateful to be alive as a result of someone else's generosity and selflessness.

STORY OF DISCOVERY

Flaws in Protein Processing: Insights from Alpha-1 Antitrypsin Deficiency

Inherited deficiencies in just a single protein can be devastating to the liver and the lungs. This protein is alpha-1 antitrypsin, or AAT. Secreted into the bloodstream primarily by the liver, this protein helps the body by inhibiting the activity of a group of enzymes, which have the power to destroy tissues.

In the genetic disease known as alpha-1 antitrypsin deficiency, this very important protein is impeded from doing its job. In severe cases, the AAT protein does not complete its journey from the interior to the exterior of the liver cell for secretion into the bloodstream. Rather, it forms polymers—orderly chains of AAT units—which aggregate in a place within liver cells that acts as a check-point to ensure the quality-control of proteins. The retention of polymerized AAT within the cells can then wreak damage on the liver. On the other hand, if inadequate levels of AAT reach the bloodstream, the protein cannot perform its important protective role of keeping a critical enzyme in check. If uncontrolled, that enzyme can cause lung tissue destruction that often progresses to emphysema. Thus, damage can come from either too much or too little AAT activity in key tissues.

NIH-funded research has helped to decipher the clinical manifestations and genetic underpinnings of AAT deficiency. Although the disease is caused by a single abnormal gene, its manifestations vary greatly depending upon whether a person inherits copies of the abnormal gene from one or both parents, and also on unknown genetic modifiers that affect gene expression. About 100,000 Americans have the severe form of AAT deficiency,[1] which is the most common genetic cause of liver disease in children, and also predisposes adults to chronic liver disease and liver cancer.[2] Over 100 variants of the AAT gene have been discovered and grouped into three categories based on the level of AAT in the bloodstream—normal, deficient, or virtually undetectable.

With knowledge gained from research has come an expanded understanding of AAT deficiency disease, the differences among its various forms, and development of therapeutics based on this scientific foundation. The importance of AAT was originally recognized in studies of blood proteins when, in 1963, scientists found that the blood of emphysema patients lacked sufficient amounts of AAT. In 1966, other researchers observed that patients with a particular variant of the AAT gene have a high frequency of liver disease, including neonatal jaundice and cirrhosis. In addition, the patients had high concentrations of AAT in their liver cells. These and other observations about the disease enabled fundamental research to begin uncovering underlying mechanisms—a prelude to therapeutic development.

For example, in the 1980s, an important step forward in combating AAT lung disease was the demonstration that augmenting a patient's natural levels of AAT with externally administered protein was feasible and beneficial. AAT-deficient patients achieved an increase in their blood levels of the protein following the intravenous transfusion of purified AAT from the blood of healthy individuals. This finding led to FDA approval of the augmentation drug, Prolastin, which has become the most widely used treatment for AAT lung disease. Other related therapies on the horizon are intravenous augmentation products, inhalation delivery systems, and synthetic augmentation therapies.

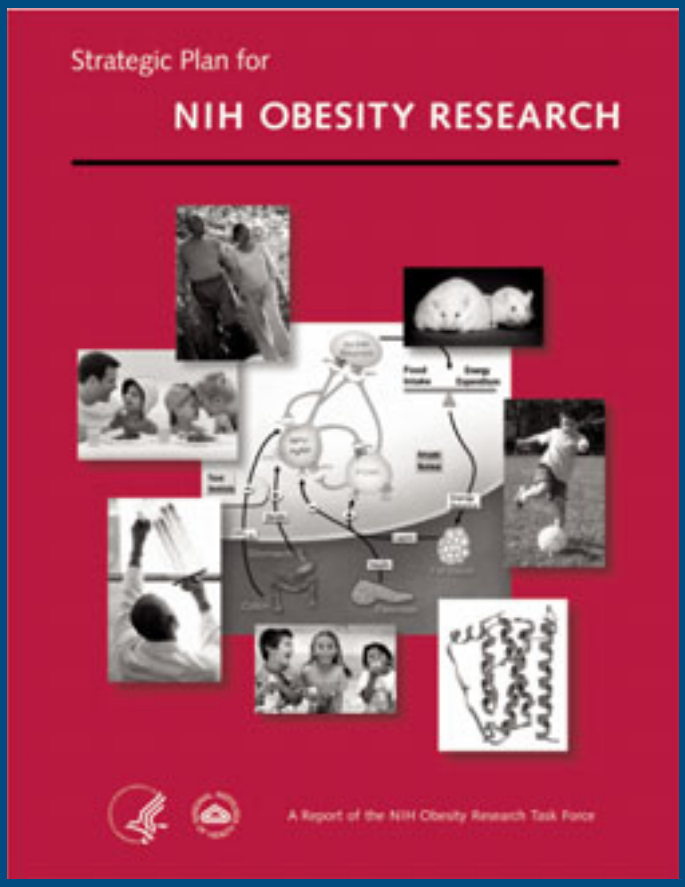
Scientists supported by the NIH have focused intense research on liver damage arising from AAT deficiency disease. Researchers have searched for factors that might predispose patients to be susceptible to or protected from this liver damage. To this end, in 1994, researchers grew skin cells from AAT-deficient individuals who had never suffered from liver disease and who therefore might be "protected." Similar cultures were made with cells from AAT-deficient individuals who had severe liver disease and were therefore considered "susceptible." While cells from both cultures accumulated the AAT protein, only the "susceptible" cells exhibited a delay in degrading AAT, suggesting that some AAT-deficient patients have alterations in the degradation pathway for the protein in their liver cells—alterations that may predispose them to developing liver disease.

Additional research supported by the NIH substantiated, in 1989, that the aggregation of AAT protein within liver cells causes liver disease. The foundation for this discovery was a study of genetically engineered mice that produced sufficient AAT to protect them from lung disease, yet the mice still suffered from a build up of AAT in their liver cells. This finding led to research aimed at improving secretion of AAT, and pointed to discrete steps in protein processing that might be used as therapeutic targets. Researchers hope to build on previous NIH supported studies in which compounds known as "chemical chaperones" have been shown to be capable of inducing AAT secretion into the bloodstream. In related research, studies have shown that patients with AAT deficiency sustain liver damage due to inflammatory immunological responses to the aggregated protein. The immunosuppressive drug cyclosporin A was shown to prevent AAT liver damage—a proof-of-principle for mechanism-based therapeutic approaches to AAT deficiency. These types of studies may lead to the testing of various drug combinations to arrest or mitigate one or more of the sequential steps in the cascade of events that culminates in liver cell injury.

A new research impetus comes from the study of small molecules that can be used therapeutically to ameliorate protein processing defects, such as those seen in AAT deficiency disease. It is expected that diseases involving abnormalities in protein processing—including AAT, cystic fibrosis, and others within the NIDDK mission—will benefit from the NIH Roadmap Initiative to develop libraries of small molecules with therapeutic potential. Knowledge about AAT deficiency and similar diseases is also being advanced by more general research conducted during the 1970s and 1980s on protein degradation. Protein processing and the degradation of old and malformed proteins are now acknowledged as essential in maintaining healthy cells. The Nobel Prize in Chemistry was awarded in 2004 in recognition of these discoveries. The NIH is proud that its sustained support of this research led to the prize-winning findings. Because AAT deficiency is an inherited disease, researchers also continue to pursue potential therapeutic approaches that could correct the genetic abnormality responsible for deficiencies in the protein. These approaches include laboratory studies of gene therapy and gene repair, as well as stem-cell approaches. The inclusion of AAT deficiency in the recently funded Cholestatic Liver Disease Consortium, jointly funded by the NIDDK and the Office of Rare Diseases, provides an opportunity to gather clinical and biochemical data and an adequate number of biosamples in a prospective manner to stimulate research on the pathogenesis and optimal diagnosis, as well as chemoprevention and treatment of this disease. The rich variety of therapeutic approaches to AAT deficiency reflects both the complexity of the disease and the expanding possibilities for multiple types of interventions to arrest or ameliorate its underlying processes.

[1] Sandhaus RA. Alpha1-antitrypsin deficiency - 6: New and emerging treatments for alpha1-antitrypsin deficiency. *Thorax* 59: 904-909, 2004.

[2] Perlmutter DH. Liver injury in alpha1-antitrypsin deficiency: an aggregated protein induces mitochondrial injury. *J Clin Invest* 110: 1579-1583, 2002.



The *Strategic Plan for NIH Obesity Research*, published in August 2004, is intended to serve as a guide for coordinating obesity research activities across the NIH and for enhancing the development of new efforts based on identification of areas of greatest scientific opportunity and challenge. It was developed by the NIH Obesity Research Task Force with critical input from external scientists and the public. The *Strategic Plan* is posted on the Task Force's Website, *http://obesityresearch.nih.gov/index.htm*. The Website also lists current NIH obesity research funding opportunities for investigators, and links to NIH health information Websites for the public and healthcare professionals.

Obesity

Obesity has risen to epidemic levels in the U.S. Obese individuals suffer devastating health problems, face reduced life expectancy, and experience stigma and discrimination. A strong risk factor for type 2 diabetes, obesity is also associated with other health conditions within the NIDDK's mission, including, for example, urinary incontinence, gallbladder disease, and the fatty liver disease non-alcoholic steatohepatitis.

Nearly 31 percent of U.S. adults are considered obese based on body mass index (BMI), a measure of weight relative to height.[1] Furthermore, while obesity and overweight have risen in the population in general, the greatest increases observed over approximately the past two decades have been in the prevalence of extreme obesity; those who are severely obese are most at risk for serious health problems.[2] Levels of childhood overweight have also escalated in the past several decades; approximately 16 percent of children and teens ages 6 through 19 are now overweight.[1,3] The levels of pediatric overweight have ominous implications for the development of serious diseases both during youth and later in adulthood. Overweight and obesity also disproportionately affect racial and ethnic minority populations, and those of lower socioeconomic status.

The increased prevalence of obesity in the U.S. is thought to result from the interaction of genetic susceptibility with behavior and factors in the environment that promote increased caloric intake and sedentary lifestyles. Thus, the NIDDK has been supporting a multidimensional research portfolio on obesity ranging from basic studies to large clinical trials. This research includes, for example, investigations to elucidate the hormones and signaling pathways that influence appetite and energy expenditure; exploration of genetic factors that predispose individuals to obesity; studies of nutrition, including diet composition; research encompassing physical activity; and studies aimed toward obesity prevention through the development and testing of modifications of environmental factors in schools, the home, and other settings. The NIDDK additionally supports research on eating disorders that are associated with obesity in some people. Highlights of recent advances from NIDDK-supported research on obesity are provided in this chapter. To help bring the results of research to the public and health care providers, the NIDDK also sponsors education and information programs.

Given the importance of the obesity epidemic as a public health problem, and its relevance to the mission of the NIDDK, the Institute has played a leading role in the NIH Obesity Research Task Force. Established by the NIH Director and co-chaired by the Directors of the NIDDK and the National Heart, Lung, and Blood Institute, the Task Force also includes representatives from numerous other NIH Institutes, Centers, and Offices. A major effort of the Task Force has been the development, with extensive input from external scientists and the public, of the *Strategic Plan for NIH Obesity Research*, published in August 2004 (*http://obesityresearch.nih.gov/About/strategic-plan.htm*).

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[1] Statistics Related to Overweight and Obesity. NIH Publication No. 03-4158, July 2003. *http://win.niddk.nih.gov/statistics/index.htm*; Hedley et al. 2004. *JAMA* 291: 2847-50.

[2] Flegal et al. 2002. *JAMA* 288: 1723-7; Flegal and Troiano. 2000. *Int. J. Obes Relat Metab Disord* 24: 807-18; Freedman et al. 2002. *JAMA* 288:1758-61.

[3] This document uses the terms overweight and obesity interchangeably for children and adolescents because there is no generally accepted definition for obesity, as distinct from overweight, in this age group.

STRATEGIC PLAN FOR NIH OBESITY RESEARCH

The *Strategic Plan* is intended to serve as a guide for coordinating obesity research activities across the NIH and for enhancing the development of new efforts based on identification of areas of greatest scientific opportunity and challenge. The *Strategic Plan* seeks to maximize collaboration among the NIH components and to capitalize on their expertise and interest in developing obesity research initiatives.

Reflecting a dynamic, trans-NIH planning process, the *Strategic Plan* presents a multi-dimensional research agenda, with an interrelated set of short-, intermediate- and long-term research goals, and strategies for achieving the goals. It builds upon a foundation of knowledge from past NIH-supported scientific advances. These advances range from the discovery of the hormone leptin, which ignited the field of molecular research into appetite control and body weight regulation, to the results of behavioral studies that led to the success of the Diabetes Prevention Program, a clinical trial demonstrating that moderate weight loss and exercise can dramatically reduce the risk of type 2 diabetes in persons at high risk for development of this disease. The major scientific research themes around which the *Strategic Plan* is framed include the following:

- Preventing and treating obesity through lifestyle modification;
- Preventing and treating obesity through pharmacologic, surgical, or other medical approaches;
- Breaking the link between obesity and its associated health conditions—including type 2 diabetes, heart disease, certain cancers, and many other health conditions;
- Cross-cutting topics, including health disparities, technology, fostering of interdisciplinary research teams to bridge the study of behavioral and environmental aspects of obesity and the study of genetic/biologic factors, investigator training, translational research, and education/outreach efforts.

Through the efforts described in the *Strategic Plan for NIH Obesity Research*, the NIH will strive to bolster progress in obesity research to improve public health.

Highlights of New NIDDK Obesity Research Initiatives: The NIDDK is pursuing a range of research avenues that will help meet the goals of the *Strategic Plan*. Critical among these is vigorous support of investigator-initiated research projects. Following are examples of efforts initiated by the Institute, in consultation with the external scientific and lay communities.

Basic Research

Several efforts will advance the understanding of the fundamental biological processes that lead to obesity. For example, the NIDDK is spearheading the development of an initiative to further understand the neurobiological basis of obesity. The NIDDK has launched an effort to bolster genetic studies of obesity-related traits in model organisms, and is planning a future endeavor to accelerate the search for obesity-related genes from human samples. The Institute is also developing a new initiative to enhance mechanistic studies of the impact of the intrauterine and neonatal environment on obesity and diabetes in offspring. Examples of research activities in other areas include the NIDDK's collaboration on an NHLBI-led initiative on bioengineering approaches to energy balance and obesity, and NIDDK's participation in an effort, led by the National Cancer Institute, to advance research on the economics of diet, activity, and energy balance.

Bridging Basic and Clinical Research

The NIDDK is pursuing a multipronged approach to promoting partnerships between basic and clinical researchers in obesity in order to propel new scientific advances. Two recent initiatives are capitalizing on major ongoing NIH research investments by soliciting ancillary studies to several existing obesity-related clinical trials and networks. A third effort independently encourages productive partnerships between basic and clinical researchers.

For the efforts focused on ancillary studies, NIDDK trials and networks that would accept meritorious obesity-related ancillary studies include:

- *Look AHEAD (Action for Health in Diabetes)*, a multi-site clinical trial which will examine the health effects of an intervention to achieve and maintain long term weight loss, through physical activity and decreased caloric intake, in 5,000 obese adults with type 2 diabetes;
- *Diabetes Prevention Program Outcome Study (DPPOS)*, described in this document in the chapter on diabetes, endocrinology, and metabolic diseases;
- *Treatment Options for Type 2 Diabetes in Adolescents and Youth (TODAY)* study, described in this document in the chapter on diabetes, endocrinology, and metabolic diseases;
- *NASH (nonalcoholic steatohepatitis) Clinical Research Network*, established to study this liver disease, which is associated with obesity;
- *Longitudinal Assessment of Bariatric Surgery (LABS)* clinical research consortium;
- *Program to Reduce Incontinence by Diet and Exercise (PRIDE)*, which will examine the impact of weight loss on urinary incontinence in overweight and obese women.

Another trial that would accept ancillary studies is sponsored by the National Institute on Aging and focuses on caloric restriction.

The NIDDK is pursuing research on long-term weight maintenance through a solicitation to encourage both basic and clinical studies in this area. With another new effort, the NIDDK is encouraging research on diet composition and energy balance to understand how different aspects of foods affect food intake, energy expenditure, and weight change.

Childhood Obesity

To bolster research on the urgent health problem of childhood obesity, the NIDDK is encouraging new studies to explore site-specific strategies for preventing and treating childhood obesity, as part of the efforts of the NIH Obesity Research Task Force. Sites encompassed by this initiative, for which meritorious research projects would be supported, include the family/home, day-care or pre-school, school, or other appropriate community venues. A complementary Task Force initiative encourages research to prevent or treat obesity in primary care settings.

New NIH Obesity Research Website: The NIDDK led the efforts of the NIH Obesity Research Task Force to develop a new obesity research Website, which was launched last year; the URL is *http://obesityresearch.nih.gov*. The primary purposes of this Website are to help inform investigators about current NIH funding opportunities for obesity research, to provide information on NIH-sponsored scientific meetings relevant to obesity, and to provide other information relevant to obesity research. In providing this information, the Website will reflect the dynamic and ongoing planning process for obesity research at the NIH. *The Strategic Plan for NIH Obesity Research* is also posted on the site. Finally, although the focus of the Website is on research, the site also includes links to other NIH Websites that provide information to the public and health professionals on weight loss, nutrition, physical activity, and health problems associated with obesity.

RESEARCH ADVANCES

Recent NIDDK-supported advances in research on obesity range from basic studies of the brain, fat tissue, and gut; to a study of teens and fast food; to assessments of potential intervention approaches.

Appetite-suppressing Hormone Rewires Brain Circuitry: The hormone leptin suppresses food intake and helps regulate body weight by communicating signals from fat cells to a part of the brain known as the arcuate nucleus of the hypothalamus (ARH). Recent studies have now shown that leptin is also fundamentally involved in developing the neural circuits in the brain that control feeding. The studies compared normal mice with mutants that are obese because they cannot produce leptin.

Distant brain cells communicate with each other by relaying electrical messages via long, wire-like connections called axons. In leptin-deficient mutant mice, the density of axons growing from the ARH was low, suggesting that one of the roles for leptin in normal mice is to promote axon outgrowth. In support of this, treating juvenile mutant mice with leptin during a critical window of time in their development restored normal patterns of brain growth. In another study, researchers found a method of distinguishing between the brain cells that control hunger and those that control satiety in a way that would permit assessment of leptin's effects on axons reaching these cells from elsewhere in the brain. Leptin-deficient mice exhibited an imbalance in physical and electrical input connections of these populations of leptin-sensitive cells. However, after just six hours of leptin treatment, the brains in the mutant mice were able to rewire and form new connections; these changes in the brain preceded observed changes in feeding behavior. Taken together, these studies mark the beginning of new and exciting advances that merge obesity research with neurobiology to demonstrate a new role for leptin in controlling the body's energy balance by regulating both long-term connections and dynamic changes in the brain.

Pinto S, Roseberry AG, Liu H, Diano S, Shanabrough M, Cai X, Friedman JM, and Horvath TL. Rapid rewiring of arcuate nucleus feeding circuits by leptin. *Science* 304: 110-115, 2004.

Bouret SG, Draper SJ, and Simerly RB. Trophic action of leptin on hypothalamic neurons that regulate feeding. *Science* 304: 108-110, 2004.

Cells of the Immune System Accumulate in the Fat Tissue of People Who Are Overweight: More than 65 percent of U.S. adults are overweight or obese, with nearly 31 percent of adults—over 61 million people—meeting criteria for obesity. Some studies have found that obese and overweight individuals have elevated levels of certain compounds in the blood that are typically observed in cases of chronic, low-grade inflammation. However it has been unclear whether these compounds result from inflammation of a single discrete part of the body, or whether they are system-wide in origin. In any case, a better understanding of obesity-related inflammation may be valuable for improving treatment for overweight patients. Using DNA microarray technology that allowed them to probe a vast array of genomic elements, researchers recently identified genes that are turned on at higher levels in fat tissue of obese mice, as compared to lean mice. Many of these genes turned out to be those that are turned on in macrophages, which are cells that contribute to the immune response, in part by inducing inflammation. Indeed, they next observed that the number of macrophages in fat tissue increased in proportion to the weight of the mouse. When they examined cells from samples of fat tissue in humans, they found a similar correlation: about 10 percent of the cells in fat samples from lean people were identified as macrophages, whereas 40 percent of the cells were macrophages in fat samples obtained from severely obese subjects. These results suggest that the cellular functions of macrophages in fat tissue may play a role in obesity and its associated disorders, and may be important therapeutic targets as well.

Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, and Ferrante Jr, AW. Obesity is associated with macrophage accumulation in adipose tissue. *J Clin Invest* 112: 1796-1808, 2003.

Statistical references: Statistics related to overweight and obesity. NIH Publication No. 03-4158, 2003. (*http://win.niddk.nih.gov/statistics/index.htm*); Hedley et al. JAMA 291: 2847-2850, 2004.

Gut Bacteria and Fat Storage: While some researchers are elucidating the complex network of hormones that control appetite and energy expenditure, and others are investigating behavioral and environmental factors that promote excess calorie consumption and sedentary lifestyles, one group of scientists is pursuing quite a different area of research toward increased understanding of obesity—gut bacteria. An enormous number of microorganisms normally reside in the gut.

Collectively referred to as the gut microbiota, these bacteria do not cause disease as would, for example, food-borne pathogens; rather, they exist relatively peacefully, and help digest various foods that their "host" human (or animal) would otherwise not be able to digest. In experiments comparing conventionally-raised mice to those raised in special laboratory conditions without microorganisms (germ-free), the scientists discovered that conventionally-raised mice contain more body fat than their germ-free counterparts. When previously germ-free mice were given gut microorganisms, they dramatically increased their total body fat content—even while decreasing their food consumption. The scientists then found that the mice also developed insulin resistance, a condition often associated with obesity and that can lead to the development of type 2 diabetes. In experiments designed to uncover the molecular mechanism for this fat storage, the scientists learned that the gut microbiota can increase the uptake of certain sugars from the gut, hence increasing the amount of calories harvested from the diet, and can boost the production of liver enzymes involved in fat production. Further, they found that in germ-free mice, a protein called Fiaf is induced; Fiaf reduces the storage of fats in fat cells. Gut microbiota suppress Fiaf, however, consequently increasing fat storage. From these studies, the scientists suggested that the gut microbiota is an important "environmental" factor that influences dietary energy acquisition and fat storage, and that increasing Fiaf activity may promote leanness.

Backhed F, Ding H, Wang T, Hooper LV, Koh GY, Nagy A, Semenkovich CF, and Gordon JI. The gut microbiota as an environmental factor that regulates fat storage. *Proc Natl Acad Sci USA* 101: 15718-15723, 2004.

Teens, Fast Food, and Obesity: As more children and adolescents have become overweight in the past several decades, one often-cited contributing factor is "fast food." Yet, while some teens who regularly eat fast food are overweight, others remain lean. In a study to try to understand why, investigators observed that lean adolescents—unlike those who were overweight—seemed to compensate for fast-food calorie intake. That is, the total amount of calories (or energy) the lean adolescents reported consuming on days when they ate fast food was not different from their caloric intake on days when they ate other types of food. In the first part of this study, the researchers offered a fast food meal, in a food court, to 54 teen participants who were age 13 to 17 years and who normally ate at least one fast food meal per week. The food available to the teens consisted of typical fast food fare, and the investigators provided as much food as each participant wanted. Both the overweight and lean adolescents overate, consuming far more calories in one meal than would be necessary for their energy needs, given that they would also be eating other meals on that day. Furthermore, the overweight participants consumed more calories than those who were lean. In the second part of the study, the researchers interviewed the teens on each of several days to ask about their diet on the preceding day. Based on what the study participant had eaten, the researchers then classified those days as either "fast food days" or "non-fast food days." The overweight teens reported consuming significantly more calories on fast food days than on non-fast food days. Although it is not clear whether such eating patterns were a cause of overweight or were secondary to weight gain, the researchers suggest that fast food consumption may at least help maintain or exacerbate obesity in susceptible individuals. By contrast, the lean study participants reported consuming a similar amount of calories on both types of day. Thus, the researchers concluded that the lean adolescents may be compensating, in their overall eating habits, for the excess calories of a typical fast food meal. The researchers cautioned, however, that effects of fast food on diet quality would suggest that such food may not be without potential detrimental effects in lean adolescents.

Ebbeling CB, Sinclair KB, Pereira MA, Garcia-Lago E, Feldman HA, and Ludwig DS. Compensation for energy intake from fast food among overweight and lean adolescents. *JAMA* 291: 2828-2833, 2004.

Liposuction Does Not Improve Risk Factors for Diabetes and Coronary Heart Disease: Liposuction is a common surgical procedure that removes substantial amounts of fat from specific areas of the body including the abdomen, hips, and thighs. Researchers have now demonstrated that liposuction to decrease fat mass in obese individuals is not an effective approach to reduce risk factors for developing serious diseases associated with obesity such as type 2 diabetes and coronary heart disease. In a study of 15 obese women, seven of whom had type 2 diabetes, researchers evaluated key obesity-associated risk factors for heart disease and diabetes prior to and 10 to 12 weeks following abdominal liposuction. The risk factors included insulin action in fat, muscle, and liver tissues, levels of certain circulating blood inflammatory proteins, cholesterol levels, blood pressure, measures of different types of body fat, and other factors. Based on these risk factors, liposuction did not provide any health benefit to either group, even though it decreased the volume of fat beneath the skin of the abdomen by 44 percent in those without diabetes and 28 percent in those with diabetes. In comparing liposuction with other weight-loss treatments which do improve metabolic risk factors associated with heart disease and diabetes—the investigators noted that liposuction removes subcutaneous fat but does not affect energy balance, that is, the balance between calories eaten and calories the body burns. By contrast, conventional diet and exercise decrease fat mass in different locations, including the fat that surrounds body organs, and creates a "negative" energy balance, which results in weight loss. The research indicates that, although liposuction removes substantial amounts of fat from beneath the skin, it alone is not sufficient to protect against obesity-associated diseases. Thus, conventional weight-loss regimens, such as diet and exercise, should be employed for effective improvement of the status of diabetes and coronary heart disease risk factors.

Klein S, Fontana L, Young VL, Coggan AR, Kilo C, Patterson BW, and Mohammed BS. Absence of an effect of liposuction on insulin action and risk factors for coronary heart disease. *N Engl J Med* 350: 2549-2557, 2004.

Intervention Prevents Excessive Weight Gain During Pregnancy in Low Income Women: Excessive gestational weight gain can have deleterious effects on both mother and child, such as complications during pregnancy, increased risk of cesarean delivery, and high infant birth weight. Researchers have tested a two-part intervention for its effect on preventing excessive gestational weight gain. The first part consisted of a clinical component, in which the women's health care providers used new tools (such as a gestational weight gain grid) to provide guidance about monitoring weight gain. In the second part, the women received patient education materials by mail. The researchers tested the intervention on women who were either overweight or normal weight at early pregnancy, and followed them until one-year postpartum. Overall, the intervention did not have any effect on preventing excessive gestational weight gain or preventing weight retention at one-year postpartum. However, when the researchers analyzed a low-income subgroup of women, they observed that the intervention effectively prevented excessive weight gain in both the overweight and normal weight low-income women; it also effectively prevented one-year postpartum weight retention in overweight, low-income women. Previous studies have shown that low-income women are at increased risk for excessive gestational weight gain. Therefore, the researchers have identified a successful intervention for this high-risk group of women.

Olson CM, Strawderman MS, and Reed RG. Efficacy of an intervention to prevent excessive gestational weight gain. *Am J Obstet Gynecol.* 191: 530-536, 2004.

The Molecular Physiology of the Control of Body Weight

Dr. Rudolph Leibel

The NIDDK National Advisory Council meets three times annually to provide advice to the Institute regarding its research portfolio and broad issues of science policy. These meetings are also an opportunity for the Council members to learn about recent scientific advances in different fields through presentations from NIDDK-supported extramural scientists. At one of the meetings in 2004, the Council and NIDDK staff were privileged to hear from Dr. Rudolph Leibel. The "Scientific Presentation" in this chapter is meant to capture the essence of his talk.

Rudolph Leibel, M.D., is Professor of Pediatrics and Medicine and Head of the Division of Molecular Genetics at Columbia University College of Physicians and Surgeons. He is also a member of the Institute of Medicine of the National Academies. A graduate of Colgate University, Dr. Leibel received an M.D. from Albert Einstein College of Medicine. His research focuses on the molecular physiology of the regulation of body weight in rodents and humans, and on the genetics of type 2 diabetes mellitus. Current research activities include efforts to identify genes (and relevant genetic variants) related to obesity and/or type 2 diabetes in mice and humans. Dr. Leibel's laboratory is dedicated to efforts to use basic, clinical, and translational research to understand human disease.

Dr. Leibel emphasized the dramatic recent increase in obesity in the United States, and its serious health consequences. In parallel to the rising rates of obesity, there has been an escalation in the levels of type 2 diabetes, for which obesity is a major risk factor. Other diseases for which obesity is a contributing factor include gallbladder disease, hypertension, and coronary heart disease, and cancer. Extensive research is shedding light on the biological control of body weight; however, finding ways to prevent and treat obesity remains challenging.

Biological Basis for the Control of Body Weight

While there are molecular controls over body weight, the body's innate regulatory defenses against loss of fat are apparently stronger than its defense against weight gain. Why is body weight (fat mass) regulated? With respect to maintaining a specific minimal level of fat, Dr. Leibel offered two answers to this question. First, a certain amount of fat (energy) is required for reproduction, including the ability to carry pregnancy to completion. Second, from an evolutionary perspective, stores of fat would have conferred protection against the environmental vicissitudes faced by our ancestors, in times when food supply was often limited. How is body weight regulated? In substantial part, this regulation is coordinated in the brain. There is a close relationship between these molecular regulatory processes and the behaviors critical to energy balance: energy intake (eating) and energy expenditure.

Numerous genes for hormones and other molecules involved in body weight regulatory pathways have been identified in mice. Scientists have also identified the human counterparts of these mouse genes, helping to elucidate the molecular control of energy balance in humans and also demonstrating the value of animal models in obesity research.

Image courtesy of the Jackson Laboratory.

Over time, a very small, persistent imbalance of energy intake over energy expenditure can lead to substantial weight gain; for example, Dr. Leibel noted that the frequent experience of gaining approximately 30 pounds (15 kg) in about 30 years, as in middle age, reflects only a 3.6 percent excess of energy intake over expenditure. From a research perspective, it will be important to develop tools to measure energy intake and expenditure with sufficient precision and accuracy to identify the nature of such small energy imbalances.

The extraordinarily complex regulatory system that controls body weight converges in a part of the brain called the hypothalamus. The brain receives signals from the blood and other organs and tissues, and it also produces molecules that can affect energy intake and expenditure. For example, the hormone leptin, secreted by fat cells, stimulates brain molecules that lead to reduced food intake, while the hormone ghrelin, produced in the gastrointestinal tract, acts to stimulate brain molecules that help drive food intake. Numerous genes for the hormones and other molecules involved in these body weight regulatory pathways have been identified in mice. Scientists have also identified the human counterparts of these mouse genes, helping to elucidate the molecular control of energy balance in humans and also demonstrating the value of animal models in obesity research. Several rare forms of human obesity result from inactivating mutations in genes that encode components of this regulatory system, including leptin and others, such as a molecule that derives from a protein called proopiomelanocortin (POMC), and the melanocortin 4 receptor, which interacts with one of the POMC-derived molecules.

A rare form of obesity—the Prader-Willi syndrome—results from a different type of genetic abnormality. Scientists have identified a small chromosomal region that is associated with this syndrome, and are working to pinpoint the genes involved. Bardet-Beidl Syndrome, another rare form of obesity, has been associated with eight different genes. A connection among several of these genes—components of a molecular structure on some brain cells—was revealed recently as a result of experiments with the corresponding genes in worms.

More common forms of obesity also have a strong genetic component. Based on studies of twins, scientists estimate that 40 to 60 percent of susceptibility to obesity is attributable to genes. For example, different people gain different amounts of weight when overfed by a specified amount, but identical twins—who share the same genes—gain similar amounts of weight. The genetic bases of common forms of obesity, however, are very complex, and susceptibility is likely influenced by many genes. Numerous regions of the genome have been implicated as containing genes that regulate body weight, and scientists are currently trying to identify these genes. Why should we have all these genes that tend to protect body weight? Genes that promote energy storage were, at one point early in human history, likely beneficial. However, in the current environment of plentiful food, such a genetic makeup leaves one prone to obesity.

Why Is Obesity So Hard To Prevent or Treat?

Dr. Leibel explained that obesity prevention and treatment are difficult not only because of the environment, but also in part because a loss of weight (fat mass) triggers the body to make compensatory adjustments in its energy expenditure that favor weight regain. That is, formerly obese individuals—those who have lost fat mass—actually require fewer calories to maintain their new weight than do individuals of the same weight who were never obese. The consequences of such weight loss, seen in patients, can include not only a slowing of their metabolic rate (energy expenditure), but also decreased satiety, increased hunger, increased work efficiency of skeletal muscles (i.e., the muscles can do more with less energy), a state of infertility, and other metabolic effects. One explanation of this phenomenon is that each individual may have a threshold for the action of leptin—a key hormone produced by fat cells, as noted previously. Each person's threshold is set by subtle sequence variations in his or her genes. When fat mass is decreased by weight loss, insufficient leptin is produced to cross this threshold.

In a clinical study that Dr. Leibel and his colleague Dr. Mike Rosenbaum conducted, individuals who had lost 10 percent of their body weight, an amount sufficient to bring about substantial health benefits, were administered just enough leptin to restore pre-weight-loss levels of the hormone. This extra leptin apparently "tricked" the brain into thinking the fat was still there, as it reversed many of the compensatory changes that normally accompany weight loss, including decreased energy expenditure.

Given these biological factors—genetics, the innate regulatory system for energy balance, and the body's natural compensatory responses to lost weight that promote weight regain—along with the current environment, losing weight, and especially maintaining weight loss are extremely difficult. The health benefits of modest weight loss are quite striking, but current treatment strategies are clearly not ideal, as reflected in very high rates of relapse to obesity.

Future Research Directions

Dr. Leibel concluded with a discussion of future research directions towards obesity prevention and treatment. The continued identification of molecules involved in body weight regulation will be important, as these could serve as new targets for drug development. It will be valuable to assess the effects of public health approaches that reduce the use of calorie-dense foods and increase physical activity. Another area for future study is research on body weight in children, including determining the optimal timing for intervention to prevent or reverse obesity. Various types of genetic approaches will enhance understanding of different forms of obesity, as well as type 2 diabetes. Finally, the development of new molecular diagnostics should help to evaluate susceptibility and to assist in the selection of therapies—which, although limited now, will hopefully be improved with continued research.

Weight-loss Surgery: Assessing Its Role in the Treatment of Obesity

Weight loss can be tough for anyone and for someone who is obese it can be a particularly daunting challenge. Moreover, for people who are extremely obese, expected weight loss from behavior change alone may not be sufficient to have a major impact on health and is unlikely to be sustained. Bariatric surgical procedures, which restrict stomach size and/or lead to decreased absorption of nutrients, are being increasingly performed to treat severe obesity. These procedures can have dramatic benefits—such as sustained weight loss, improved control of blood sugar levels, or even reversal of type 2 diabetes—especially when accompanied by a healthy diet and exercise. However, they also carry substantial risks, including death in a small number of patients.

Despite the increasing popularity of bariatric surgery, crucial questions still remain, such as how best to identify candidates for surgery, and the extent of potential health benefits *versus* potential risks. Researchers would also like to figure out precisely how certain types of bariatric surgery work to help patients maintain weight loss or to improve obesity related diseases. To address many of these questions, the NIDDK is building upon recent advances and emerging opportunities as a foundation for new research efforts—including a major new clinical research initiative.

What Is Bariatric Surgery?

In recent years, bariatric surgery, also called weight-loss surgery, has garnered a lot of media attention. However, the first surgery of this type used for severe obesity actually dates back 40 years and grew out of the results of operations for certain cancers or severe ulcers. Doctors became aware that their patients lost weight following surgeries that removed large portions of the stomach or small intestine. Some physicians began to use such operations to treat patients with severe obesity. Over time, these operations have been modified to improve patient safety and to incorporate technological advances in surgical procedure. Today, there are basically two types of bariatric operations: restrictive and malabsorptive.

In restrictive operations, physicians use surgical staples and/or a special band to create a small pouch at the top of the stomach at the point that food enters from the esophagus. These operations restrict food intake but do not interfere with the normal digestive process. Patients lose the ability to eat large amounts of food at one time. Although restrictive operations lead to some degree of weight loss in almost all patients, these surgeries are less successful in inducing sustained weight loss. This result is largely because patients must still adjust their diets to reduce total caloric intake, and can “out eat” their surgery with frequent small portions of liquid calories or easily absorbed foods.

Malabsorptive operations, on the other hand, restrict both food intake and the amount of calories and nutrients the body absorbs, and are by far the most common gastrointestinal surgeries for weight loss. The most commonly performed operation, which has both a restrictive and malabsorptive component, is the Roux-en-Y Gastric Bypass. These procedures connect the upper stomach to the lower part of the small intestine, so that food bypasses a large portion of the gastrointestinal tract in which digestion and nutrient absorption normally take place. Patients who have this type of surgery generally lose two-thirds of their excess weight within two years, and tend to keep much of it off for years. The tradeoff for patients is a greater risk of developing nutritional deficiencies, especially in iron and calcium. Other malapsorptive procedures, such as the biliopancreatic diversion with or without duodenal switch, produce even more dramatic weight loss, but at the expense of more complications, including nutritional deficiencies.

Who Is Having Bariatric Surgery?

The prevalence of overweight and obesity has risen dramatically in the United States over the past several decades. Even more alarmingly, the prevalence of extreme obesity has risen at a faster pace. One widely used measure of excess weight is the body mass index (BMI), a ratio of a person's weight in kilograms divided by the square of his or her height in meters. Between the period 1988 to 1994 and the period 1999 to 2000, the age-adjusted prevalence of obesity among adults in the nation, as measured by BMI, rose from 55.9 to 64.5 percent; however, the prevalence of extreme obesity—a BMI of 40 or more—rose in the same period from 2.9 to 4.7 percent.

The trend of increased obesity in the U.S. population has been paralleled by an increase in the number of bariatric surgeries performed: According to the American Society for Bariatric Surgery, the number of operations increased from about 16,000 in the early 1990s to more than 103,000 in 2003.

The popularity of bariatric surgery arises from its ability to overcome the major challenges of treating extreme obesity: bariatric surgery is currently the most effective means to induce substantial weight loss and to maintain that loss. However, bariatric surgery is not a cosmetic procedure, but a major, serious operation requiring lifelong adjustments in diet and behavior to ensure success. Moreover, adults who are candidates for these procedures are usually not in the best of health. They have a BMI of 40 or more—the equivalent of 80 to 100 pounds or more of excess weight—or a BMI of 35 or more and serious obesity-related complications, such as cardiopulmonary problems, type 2 diabetes, severe sleep apnea, or joint problems. These conditions place bariatric surgery candidates at high risk for death or complications, either from their obesity-related medical problems, or from the surgery itself. Thus, the benefits and risks of bariatric surgery need to be carefully assessed before an already vulnerable patient embarks on this challenging course of treatment for obesity and its complications.

KNOWN BENEFITS AND RISKS OF BARIATRIC SURGERY

Benefits of Bariatric Surgery

- Bariatric surgery generally leads to weight loss, with results varying depending upon the type of surgery selected. For example, between 18 to 24 months after malapsorptive surgery most people lose two-thirds of their excess weight, much of which is sustained over many years.
- Bariatric surgery leading to weight loss improves most obesity-related conditions, including diabetes, high blood pressure, lipid abnormalities, and sleep apnea.

Risks of Bariatric Surgery

- 10 to 20 percent of patients require follow-up operations to correct complications, most commonly abdominal hernias.
- Some patients develop gallstones, which are clumps of cholesterol and other matter that form in the gallbladder. However, taking supplemental bile salts can prevent gallstones from developing.
- Nearly 30 percent of patients develop nutritional deficiencies such as anemia, osteoporosis, and metabolic bone disease, which can be avoided by taking sufficient amounts of vitamins and minerals.
- Women of childbearing age should avoid pregnancy until their weight becomes stable, because rapid weight loss and nutritional deficiencies can harm a developing fetus.
- Some patients experience psychological difficulties.
- Estimates of postoperative death rates for bariatric surgery patients range from 0.1 to 2 percent.

Longitudinal Assessment of Bariatric Surgery (LABS)

While bariatric surgical procedures can have dramatic health benefits, they also carry substantial risks, including death. Thus, it is very important for physicians and potential patients to have the most comprehensive information possible with which to make well-informed choices about weight-loss surgery as a means to treat extreme obesity. Until recently, this information has been limited by a paucity of systematic research that could help determine the full spectrum of risks and benefits and provide evidence-based guidance on appropriate patient selection. In particular, researchers have lacked a common database of information on patients and outcomes for healthcare professionals.

Responding to this information gap, the NIDDK has been working to advance efforts to examine bariatric surgery more carefully as a treatment option for obesity. In 2001 and 2002, the NIDDK convened groups of external experts to discuss recent developments in bariatric surgery, to identify pressing clinical questions, and to identify scientific research opportunities pertaining to bariatric surgery and its impact on obesity and co-morbid conditions. These meetings built upon earlier findings from a 1991 NIH Consensus Development Conference on bariatric surgery. The first meeting, a workshop convened in collaboration with the American Society for Bariatric Surgery, focused on clinical research issues. The workshop participants emphasized the need to determine the impact of bariatric surgery on subsequent pregnancy, the impact of age on outcomes, and the effect of operations with greater malabsorptive potential on nutritional status.

In May 2002, this effort was expanded by the NIDDK Working Group on Bariatric Surgery. Through a two-day meeting, the group identified numerous clinical and basic research opportunities related to bariatric surgery, such as using this surgery as a model to understand the underlying pathophysiology of obesity-related diseases—many of which are reversed or ameliorated following surgery. Another research opportunity is the evaluation of the safety and efficacy of bariatric surgical procedures, including their impact on weight loss, obesity-related health conditions, psychosocial status, quality-of-life, and economic factors. Studies looking at both short-term and long-term outcomes for patients were felt to be critical. Also considered essential is the refinement of the evaluation of factors that may contribute to a person's obesity (such as physiologic, metabolic, and genetic factors) to better predict outcomes, and hence improve the ability to assess the risk/benefit ratio for an individual bariatric surgery patient. The development of a patient database was recommended.

Based upon the input from external experts, the NIDDK has now established a bariatric surgery clinical research consortium, the Longitudinal Assessment of Bariatric Surgery (LABS), to facilitate and accelerate research in this area. Over the course of five years, the LABS consortium will plan, develop, and conduct coordinated clinical, epidemiological, and behavioral research in bariatric surgery, both restrictive and malabsorptive operations. This consortium will help pool the necessary clinical expertise and administrative resources to facilitate the conduct of multiple and novel clinical studies in a timely, efficient manner. Development of a database using standardized definitions, clinical protocols, and data collection instruments will enhance the ability to provide meaningful evidence-based recommendations for patient evaluation, selection, and follow-up care. This database, in turn, will promote rapid dissemination of research findings to healthcare professionals.

In addition, this consortium will serve as a resource for basic and clinical studies to explore the mechanisms by which bariatric surgery affects obesity-related health conditions, physical activity, appetite and eating behaviors, and psychosocial factors. This research may lead to improved understanding of the factors underlying the development of obesity, with implications for new strategies for prevention and treatment. For example, recent studies suggest that malabsorptive bariatric surgeries disrupt normal neural, gastrointestinal, and endocrine pathways that influence appetite, physical activity, and the sense of fullness. Researchers hypothesize that these disruptions may explain, in part, the success of malabsorptive operations in the maintenance of weight loss. Thus, studies of large numbers of persons

who have undergone bariatric surgery may also provide new insights into the role of peripheral signaling (signaling from various parts of the body to the control centers in the brain) in controlling the balance between energy intake (calories) and energy expenditure (physical activity and metabolism) that is derailed in obesity. New NIDDK-led initiatives are encouraging such research, to be performed as ancillary studies to the LABS consortium.

Enrollment for the LABS consortium is expected to begin in early- to mid-2005. Through the LABS and related research efforts, the NIDDK hopes to improve the understanding and application of bariatric surgery as a treatment for severe obesity. Although prevention remains the primary public health goal in the face of rising rates of overweight and obesity, more effective treatments are crucial for those who are already obese. Studies on bariatric surgery will likely contribute to both these objectives.

Weighing In On Weight-Loss Surgery—One Patient's Perspective

By age 43, biologist Eli Ney, at 242 pounds, was overweight by well over 100 pounds, which classified her as morbidly obese. Eli, now 45, says that over the years she had been on every type of diet imaginable, and that, on at least two occasions, she had lost 100 pounds or more, only to put them back on. "After a while the dieting became very discouraging," she says. However, two years after undergoing bariatric surgery, Eli now maintains her weight at 128 pounds—"give or take a pound or two," she chuckles. Through good eating habits and continued exercise, she intends to keep it that way. But, Eli admonishes that anybody contemplating weight-loss surgery needs to do their homework. "You had better know what you're getting yourself into before you get on that operating table," she says.

The Surgery: Eli never had it easy when it came to controlling her weight. "I've always been on a diet, even when I was a child," she says. As a very little girl she remembers always having "a pooch around my middle." Eli's family also has a health history that includes type 2 diabetes, which is often associated

Eli Ney

with being overweight. "My mother has type 2 diabetes, high blood pressure, high cholesterol, and abdominal obesity. Several years ago she had a heart attack. I saw myself heading down that path," she says.

At age 42, in lieu of attempting yet another frustrating diet, Eli decided to research the pros and cons of bariatric surgery. She decided that, for her, the potential positive outcomes were worth the known risks. In August 2001, she submitted health information to a bariatric physician—including her dieting history, current weight, and BMI. In May of 2002, she had Roux-en-Y bypass surgery, the most common form of malabsorptive surgery. The surgery initially reduced her stomach capacity from three pints to one ounce of food, a tremendous adjustment in terms of food intake. Any overeating whatsoever now can cause "dumping syndrome," which means that the contents in Eli's stomach move too rapidly through her small intestine. Symptoms include nausea, weakness, sweating, faintness, and sometimes diarrhea after eating, and it can "knock you out for hours," says Eli.

The first day or two after surgery, Eli found it difficult to get up and walk, but within 2½ weeks she was back at work. "People ask me all the time 'Do you feel different?,' but I don't, really," she says. Eli is outgoing—she has been a member of a Toast Masters speakers' club since 1998—and always has had a strong self-image. But she does acknowledge that, once she started losing weight, people began paying more attention to her. "Weight-loss can be a pretty intense experience, especially when you realize how differently you are treated," says Eli. "Some

people who wouldn't even say 'hi' to me before, do now. It's sad but true."

Benefits: Within 18 months of her surgery, Eli arrived at her current 128 pounds. The surgery also reduced most of the other obesity-related conditions she had been experiencing—including high blood sugar levels, a warning sign of impending type 2 diabetes. For Eli, however, the real test of the surgery's success came during times of personal crisis that followed closely on the heels of her operation. "As a result of my surgery, I felt empowered not to use food as a coping mechanism," she says.

Side Effects: In addition to "dumping syndrome," Eli has experienced a number of side effects from the surgery, due to the reduced absorption of nutrients. For example, Eli says she lost 25 percent of her hair because of protein loss. "It's all back now, but it's scary when you're combing your hair and large hanks of it come out in the brush." Also, since the surgery, Eli has gone from being 5-feet 3½ inches tall to 5-feet 2¾ inches. She attributes this to bone loss and osteoporosis and is careful about making sure she gets her daily supplements of calcium. As a result of the surgery, she also has mild anemia, which she counteracts with iron supplement pills, on top of a regimen that includes daily doses of multiple vitamins and a shot of B12 supplement every 6 months. Eli recently underwent reconstructive surgery to remove excess skin caused by the loss of such a large amount of weight. "For some people reconstructive surgery is mandatory. For me it was optional, but it was something I felt I needed to do to complete this process."

To this day, Eli is committed to good diet and to avoiding foods that contain sugar, including cookies, cakes or sweetened soft drinks, as well as breads and other foods high in carbohydrates. She also continues to exercise regularly. In fact, Eli is so committed to her new life that she is currently using her Toast Master speaking skills to reach out to obese men and women to explain to them their options for losing weight, and is considering writing a book about her own experience. "I've already got a title," Eli says cheerily. "Extreme Makeover from the Inside Out."

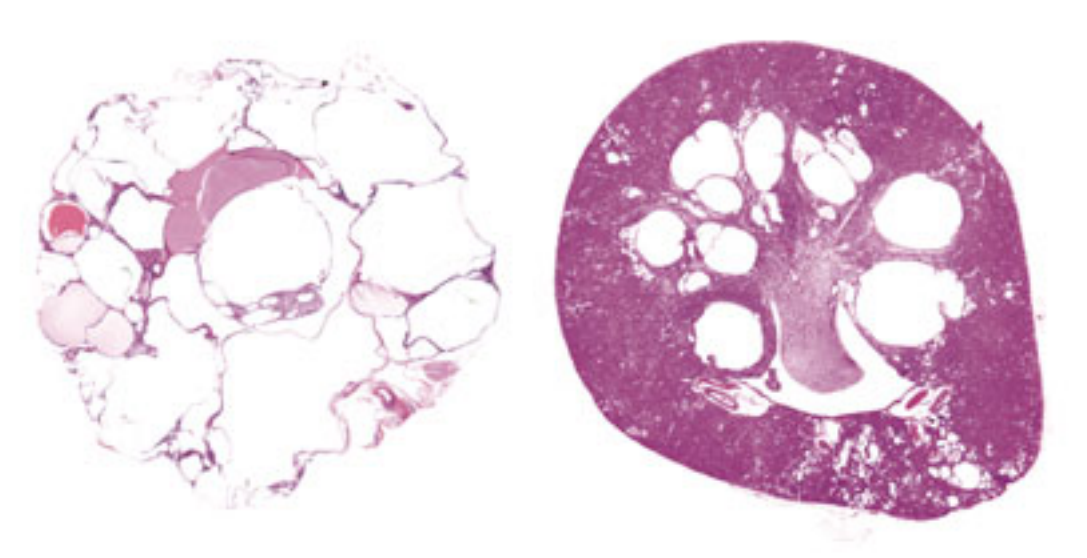
WIN: NIDDK's Weight-control Information Network

The NIDDK's Weight-control Information Network (WIN), provides the general public, health professionals, and the public with up-to-date, science-based information on obesity, weight control, physical activity, and related nutritional issues. This information includes fact sheets and brochures for the public, as well as *WIN Notes*, a periodic newsletter for health professionals and consumers. Through its information services, WIN reaches out to people of all ages, and to diverse ethnic and racial groups.

WIN is conducting several outreach activities. Among these is an ongoing project to promote the distribution by healthcare providers, including primary care providers, of information regarding food labels, regular physical activity, and how to help obese patients. Another outreach effort to community health clinics focuses on WIN's series of booklets on "Cómo Alimentarse y Mantenerse Activo Durante Toda La Vida" ("Healthy Eating and Physical Activity Across Your Life Span"). This project involves communications to physicians, dietitians, and community health centers, including Hispanic healthcare professionals and community healthcare centers that serve predominantly Hispanic populations. The "Toda La Vida" series of booklets, available in English and Spanish, contains booklets targeted to different populations, including "tips for adults," "tips for older adults," "tips for parents," and "tips for pregnancy."

WIN's "Sisters Together: Move More, Eat Better" is a national initiative that encourages African American women to maintain a healthy weight by becoming more physically active and eating more healthful foods. Among its publications are: "Celebrate the Beauty of Youth!," which was published this past year, "Fit and Fabulous as You Mature," "Energize Yourself and Your Family," and "Walking...A Step in the Right Direction." WIN is conducting an outreach effort to contact historically Black colleges and universities (HBCUs) and various community venues to promote the availability of "Sisters Together" brochures.

This past year, WIN redesigned its Website with the goal of making information regarding weight loss and control easier to find and navigate. The new address is *http://win.niddk.nih.gov*.



Left: Histologic section taken from kidneys of mice that develop kidney disease similar to polycystic kidney disease (PKD) in humans. Right: Section of a cystic kidney taken from a rat model of PKD. Scientists use animal models of diseases to study progression and evaluate potential new therapeutic approaches. Images courtesy of Dr. Vicente Torres and reprinted from Torres *et al*. *Nat Med* 10: 363-364 (2004) and Gattone *et al*. *Nat Med* 9: 1323-1326 (2003).

Kidney, Urologic, and Hematologic Diseases

Diseases of the kidneys, urologic system, and blood are among the most critical health problems in the U.S. They afflict millions of Americans, including children and young adults. The NIDDK is committed to enhancing research to understand, treat, and prevent these diseases.

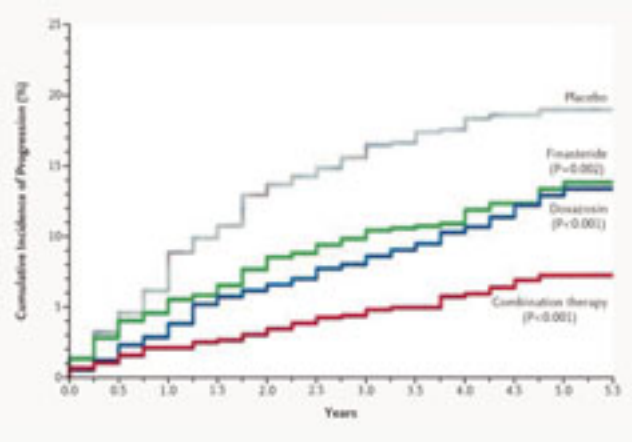
Normal, healthy kidneys filter toxins from the blood, concentrating them in urine so that they may be ultimately excreted from the body. In people with chronic kidney disease, the function of these life-sustaining organs is impaired. Kidney disease may progress to irreversible kidney failure, also known as end-stage renal disease (ESRD). People with ESRD require dialysis or a kidney transplant to live. Conservative estimates find that 4.5 percent of American adults 20 years of age and older—about eight million adults—have substantially impaired kidney function. The leading cause of kidney disease is diabetes, with hypertension (high blood pressure) the second-leading cause. The recent increases in obesity and type 2 diabetes in the U.S., if left unchecked, will have grave implications in several years, as more people begin to develop renal complications of diabetes.

Racial minorities, particularly African Americans, Hispanics, and American Indians, bear a disproportionate burden of chronic kidney disease. African Americans are four times more likely and American Indians are twice as likely to develop kidney failure than are non-Hispanic whites. Hispanics have a significantly increased risk for kidney failure, as well.

The U.S. has seen an enormous increase in the number of people with ESRD. The NIDDK-supported United States Renal Data System (USRDS), a nationwide database of kidney disease information, reports that over 100,000 people developed ESRD in 2002, the most recent year for which statistics are available. Additionally, over 400,000 patients were living with the disease at the end of that year, with more than 300,000 receiving dialysis and over 120,000 with a functioning kidney transplant. These numbers have doubled since 1990 and are expected to nearly double again by 2010.

The NIDDK supports a significant body of research aimed at increased understanding of the biology underlying chronic kidney disease. The chronic renal diseases program supports basic and clinical research on kidney development and disease, including the causes of kidney disease; the underlying mechanisms leading to progression of kidney disease to ESRD; and the identification and testing of possible treatments to prevent development or halt progression of kidney disease. Areas of focus include diseases that collectively account for more than half of all cases of treated ESRD. Of special interest are studies of inherited diseases such as polycystic kidney disease, congenital kidney disorders, and immune-related glomerular diseases, including IgA nephropathy and the hemolytic uremic syndrome. A major new educational outreach effort is the National Kidney Disease Education Program, which is designed to raise awareness among patients and physicians about the problem of kidney disease and steps that should be taken to treat chronic kidney disease and prevent kidney failure.

Urologic diseases affect men and women of all ages, result in significant health care expenditures, and—if misdiagnosed or improperly treated—may lead to substantial disability and impaired quality of life. The NIDDK's urology research portfolio includes basic and clinical research on the normal and abnormal development, structure, and function of the genitourinary (GU) tract. The NIDDK also supports studies of a number of noncancerous urologic



The MTOPS clinical trial found that combination therapy with two drugs that act through different mechanisms was more effective at preventing progression of benign prostatic hyperplasia (BPH) than either drug alone. The graph shows the cumulative incidence of progression in men with BPH who received either a sugar pill (placebo, grey line); an inhibitor of the enzyme 5-alpha reductase (finasteride, green line); a beta blocker (doxazosin, blue line); and finasteride and doxazosin together (red line). For more information, see page 74.

diseases, include benign prostatic hyperplasia, prostatitis, urinary tract infections, urinary tract stone disease, interstitial cystitis, urinary incontinence, and congenital anomalies of the GU tract.

Benign prostatic hyperplasia, or BPH, is a serious condition that is especially common among older men. Almost one-half of men over age 70 report lower urinary tract symptoms that are consistent with a diagnosis of BPH. Prostatitis—chronic inflammation of the prostate gland—is a painful condition that accounts for a significant percentage of all physician visits by young and middle-aged men for complaints involving the genital and urinary systems. The NIDDK is committed to enhancing research to understand, treat, and prevent these common and troubling disorders.

Infections of the urinary tract are extremely common in women, and many women suffer repeated urinary tract infections (UTIs). In 2000, UTIs and cystitis accounted for over nine million physician visits. Interstitial cystitis (IC) is a debilitating, chronic, painful bladder disease that has been estimated to affect as many as 847,000 American adults, over 90 percent of whom are women. Millions of Americans, most of them women, suffer from urinary incontinence. For both men and women, kidney stones, a condition formally known as urinary tract stone disease, accounted for over 2.2 million physician visits in 2000. In children, one of the most common causes of kidney failure, vesicoureteral reflux, occurs in an estimated 1-to-2 percent of newborns. In fact, abnormalities of the GU tract are the most common birth defects.

To address these and other urologic problems, the NIDDK's urology research efforts support basic, applied, and clinical research in prostate function and prostate diseases; diseases and disorders of the bladder; male sexual dysfunction; urinary tract infections; urinary tract stone disease; and pediatric urology, including developmental biology of the urinary tract. A research emphasis of the urology program is the study of chronic inflammatory disorders of the lower urinary tract.

The NIDDK's hematology research program uses a broad approach to enhance understanding the normal and abnormal function of blood cells and the blood-forming system. Research efforts include studies of a number of blood diseases, including sickle cell disease, the thalassemias, aplastic anemia, iron deficiency anemia, hemolytic anemias, and thrombocytopenia. The Institute is also keenly interested in the basic biology and genetic regulation of stem cells, especially adult hematopoietic stem cells, which are needed for bone marrow transplants and may have broader application in gene therapy research. An additional priority of the Institute's hematology research program is the development of improved iron chelating drugs to reduce the toxic iron burden in people who receive multiple blood transfusions for the treatment of diseases.

ADVANCES IN KIDNEY DISEASE RESEARCH

A Potential New Therapy for Polycystic Kidney Disease (PKD): PKD and other inherited cystic kidney diseases frequently cause kidney failure and death, often in children. There are no effective treatments. One characteristic common to several of these disorders is an elevated level of cyclic adenosine monophosphate (cAMP) in the kidneys. Within cells, cAMP transmits messages that affect their growth and function; abnormally high levels of cAMP in certain kidney cells are thought to contribute to cyst formation. Researchers treated animal models of the two predominant forms of human PKD and another cystic kidney disease using a chemical, OPC31260, which lowers cAMP production in the kidneys. The treatment halted disease progression, and in some cases resulted in improvement. OPC31260 and similar compounds are currently undergoing testing in human clinical trials for treatment of other diseases and so far appear to be safe. Thus, drugs of this class are promising candidates for phase I clinical trials to treat patients with PKD.

Torres VE, Wang X, Qian Q, Somlo S, Harris PC, and Gattone II, VH. Effective treatment of an orthologous model of autosomal dominant polycystic kidney disease. *Nat Med* 10: 363-364, 2004.

Gattone II, VH, Wang X, Harris PC, and Torres VE. Inhibition of renal cystic disease development and progression by a vasopressin V2 receptor antagonist. *Nat Med* 9: 1323-1326, 2003.

Impact of Chronic Kidney Disease on Cardiovascular Health: Of the estimated eight million Americans with chronic kidney disease,[1] more than 400,000 have end-stage renal disease, or ESRD, with over 300,000 requiring dialysis to live.[2] ESRD patients are known to have very high rates of cardiovascular disease (CVD), which kills about half of them. However, until recently, it was unknown to what degree less serious chronic kidney disease predisposes patients to develop CVD. The Modification of Diet in Renal Disease clinical trial provided strong evidence that kidney function can be reliably estimated by measuring the amount of a compound called creatinine in a patient's blood, and performing a calculation that also includes variables such as the person's size and sex. Now, researchers have built upon that finding by examining the results of creatinine tests from more than one million patients to assess kidney health and look for correlations with cardiovascular outcomes such as heart attacks. The researchers found a very clear pattern: the poorer a patient's kidney function, the more likely he or she was to develop CVD. Armed with the knowledge that kidney health is a predictor of CVD, health care providers can now determine that some of their patients are at risk, and may be more likely to benefit from earlier, more aggressive cardiovascular treatment than might otherwise have been prescribed.

Go AS, Chertow GM, Fan D, McCulloch CE, and Hsu CY. Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization. *N Engl J Med* 351: 1296-1305, 2004.

Hostetter TH. Chronic kidney disease predicts cardiovascular disease. *N Engl J Med* 351: 1344-1346, 2004.

Statistical References

[1] Coresh J, Astor BC, Greene T, Eknoyan G, and Levey AS. Prevalence of chronic kidney disease and decreased kidney function in the adult US population: Third National Health and Nutrition Examination Survey. *Am J Kidney Dis* 41: 1-12, 2003.

[2] 2004 USRDS Annual Data Report Atlas. United States Renal Data System, 2004. (*http://www.usrds.org/atlas.htm*)

Pinpointing the Location of Adult Kidney Stem Cells: The cells of a healthy adult kidney rarely divide to make new copies of themselves. However, kidneys retain a limited capacity for self-repair in case of injury. That repair requires replacing damaged cells of multiple types. This is the kind of task the body relegates to stem cells, which by definition can divide and differentiate into multiple cell types. Recent research using rodents has determined that stem cells capable of forming new kidney cell types are largely or entirely confined to a small portion of the kidney called the "renal papilla." Further research

can now proceed to determine whether these cells can form any kidney cell type, or just a subset of them. Even more importantly, researchers will be seeking the specific signals that trigger kidney stem cells to form each cell type. With this knowledge, it may one day be possible to stimulate patients' innate ability to heal their kidneys, thereby reducing the need for dialysis and kidney transplantation.

Oliver JA, Maarouf O, Cheema FH, Martens TP, and Al-Awqati Q. The renal papilla is a niche for adult kidney stem cells. *J Clin Invest* 114: 795-804, 2004.

Kidney Disease Clinical Research: The NIDDK recognizes the importance of encouraging the development of novel ideas for clinical interventions related to kidney disease. For the past two years, the NIDDK has sponsored a pilot program aimed at providing supplemental funding to NIDDK-supported investigators to encourage them to undertake such studies. The "Kidney Disease Clinical Studies Initiative" is an outgrowth of a task force meeting convened in March 2002 by the NIDDK and the Council of American Kidney Societies (CAKS). Since the inception of this initiative, the NIDDK has seen growing demand from the research community for this funding, and has developed new funding mechanisms for research concept development and ancillary studies. The NIDDK anticipates holding a follow-up meeting with the kidney research community sometime in 2005 or 2006 to discuss ways of strengthening this program. Also, plans for a program of small planning grants for clinical studies have grown out of the experience with supplemental awards.

TREATMENT STRATEGIES FOR BENIGN PROSTATIC HYPERPLASIA

Combination Therapy for Benign Prostatic Hyperplasia: Benign prostatic hyperplasia, or BPH, is a condition that affects an estimated nine percent of men 30 years of age and older. Prevalence increases significantly in middle age, with the majority of cases reported in men age 55 and older. BPH can result in frequent urination, inability to urinate, and urinary tract infections. For many years, surgery was the only viable treatment option, although new drug therapies have recently emerged from research studies. The NIH launched the Medical Therapy of Prostatic Symptoms (MTOPS) clinical trial to assess the safety and efficacy of different interventions on BPH symptoms and progression. Study participants were divided into four groups, and received either placebo (sugar pill), one of two Food and Drug Administration-approved medications for BPH with different mechanisms of action, or the two drugs in combination. The study followed participants for an average of five years and the results were striking. Although each drug was effective when used alone (the risk of BPH progression was reduced by 39 percent with one and by 34 percent with the other), the combination drug therapy reduced the risk of BPH progression by 67 percent compared to placebo. The MTOPS trial conclusively demonstrated that combination therapy is safe, and is the most effective treatment for men with symptomatic BPH.

McConnell JD, Roehrborn CG, Bautista OM, Andriole GL, Jr., Dixon CM, Kusek JW, Lepor H, McVary KT, Nyberg Jr, LM, Clarke HS, Crawford ED, Diokno A, Foley JP, Foster HE, Jacobs SC, Kaplan SA, Kreder KJ, Lieber MM, Lucia MS, Miller GJ, Menon M, Milam DF, Ramsdell JW, Schenkman NS, Slawin KM, and Smith JA for the Medical Therapy of Prostatic Symptoms (MTOPS) Research Group. The long-term effect of doxazosin, finasteride, and combination therapy on the clinical progression of benign prostatic hyperplasia. *N Engl J Med* 349: 2387-2398, 2003.

Despite advances in the medical treatment of BPH, some men will ultimately require surgery to alleviate their symptoms. For many years, transurethral resection of the prostate (TURP) has been the standard surgical therapy for this condition. With TURP, an instrument called a resectoscope is inserted up the urethra. The physician uses the resectoscope's wire loop to remove the tissue causing the obstruction of the urethra, thereby alleviating the discomfort and urinary urgency associated with BPH.

Over the past decade, a number of technical innovations have furthered the development of new

surgical treatments that aim to achieve the same long-term outcomes of TURP, but with less morbidity, lower costs, in-office treatment or shorter hospital stays, and more rapid recovery. These new, "minimally-invasive" surgical approaches include laser therapy, hyperthermia and thermotherapy, transurethral electrovaporization, microwave therapy, and transurethral needle ablation. Furthermore, newer techniques are appearing regularly. However, published reports on the outcomes of these minimally-invasive therapies are highly variable in their quality, and rigorously conducted long-term, multicenter randomized clinical trials have only rarely been conducted. To assess the long-term safety and effectiveness of these new therapies, the NIDDK has launched the MIST clinical trial. MIST, "Minimally Invasive Surgical Therapy for BPH," will evaluate the safety and effectiveness of transurethral needle ablation (TUNA) and transurethral microwave therapy (TUMT), procedures whereby radio waves or microwaves, respectively, are used to heat and destroy obstructing prostate tissue; and combined medical therapy with alfuzosin, an alpha blocker, and finasteride, an alpha-reductase inhibitor in men with BPH. The results of this trial will help to provide both physicians and patients with the knowledge needed to make the most appropriate choices for long-term management of BPH.

OPPORTUNITIES IN WOMEN'S UROLOGIC HEALTH

Urinary Incontinence: More than 13 million people in the U.S.—men and women of all ages—experience urinary incontinence. Women experience incontinence twice as often as men. Pregnancy and childbirth, menopause, and the structure of the female urinary tract account for this difference. Incontinence in women usually occurs because of problems with muscles that help to hold or release urine. One kind of urinary incontinence is stress urinary incontinence, which is the accidental leakage of urine during activities such as coughing, laughing, sneezing, or lifting heavy objects. Another type, urge incontinence, is the leakage of large amounts of urine at unexpected times, including during sleep.

To address the problem of urinary incontinence in women, the NIDDK, along with the National Institute of Child Health and Human Development and the NIH Office of Research on Women's Health (ORWH), supports a Urinary Incontinence Treatment Network (UITN). The Network is a group of urologists and urogynecologists who are investigating possible new treatments. The Network has begun a clinical trial comparing two surgical treatments for stress and mixed incontinence—the "Stress Incontinence Surgical Treatment Efficacy Results" (SISTER) trial. The SISTER study is comparing the long-term outcomes of two commonly performed surgeries for the treatment of stress urinary incontinence. A second clinical trial planned by the Network is focused on treating women with pure or predominantly urge incontinence. This trial, the "Behavior Enhances Drug Reduction of Incontinence" (BE-DRI) trial, will compare effects of two interventions—drug therapy alone and combination drug therapy and behavioral treatment—on the frequency of urinary incontinence and success in withdrawing patients from drug therapy.

In another effort aimed a treating incontinence, the NIDDK has recently funded the "Program to Reduce Incontinence by Diet and Exercise" (PRIDE) study, which will evaluate the impact of weight loss, from a behavioral program, on urinary incontinence in overweight and obese women. About 300 overweight women ages 30 or older will participate in this clinical trial.

Interstitial Cystitis: Interstitial cystitis (IC) is a chronic bladder disease characterized by pelvic pain and increased frequency and urgency in urination. These symptoms can be quite debilitating, interfering with a patient's ability to work, go out, and enjoy life. While the precise number of persons affected is unknown, as many as 847,000 American adults may suffer from IC; however, 90 percent of reported cases occur in women. The causes of IC are as yet unknown. Current treatments

for symptoms are not effective in all patients, and there is no cure. The NIDDK is supporting clinical and basic research investigations on several fronts to understand the causes of IC, to develop and test more effective treatments, to develop better diagnostic tools, and, ultimately, to develop a cure for this disease.

In October 2004, the NIDDK held a meeting of the more than 20 grantees who received research funding through a recent Request for Applications (RFA) for "Basic Research in Interstitial Cystitis." The group discussed ongoing work and heard descriptions of encouraging results and future plans for work on a promising biomarker for IC, antiproliferative factor (APF). To build on this discovery, the NIDDK is currently developing a translational research initiative that will accelerate efforts to validate APF's usefulness as a diagnostic tool for IC. The NIDDK plans to support another meeting of the investigators in 2005; this meeting will help continue the critically important cross-fertilization of ideas among researchers in the field, and will also help guide the Institute's decisions regarding support for larger meetings of IC investigators in the future.

In Fall 2004, the NIDDK initiated its new Interstitial Cystitis Awareness Campaign. This campaign will reach out to two target audiences: healthcare professionals, especially urologists, and American women between the ages of 25 and 50 who may have IC. Outreach materials for this campaign include information on symptoms, diagnostic protocols, treatment strategies and research for IC patients. The National Kidney and Urologic Diseases Information Clearinghouse is the primary distribution channel for campaign materials. In developing this important awareness campaign for patients and healthcare practitioners, the NIDDK received input from a patient-based advocacy group for IC through its participation in an *ad hoc* coordinating panel for the Clearinghouse.

Efforts to inform physicians and the public about IC will be significantly enhanced when there is a consistent and clinically useful definition. Some progress has been made in identifying common clinical symptoms among some IC patients. The NIDDK anticipates that recent efforts to review the rapidly evolving science surrounding IC will lead to a better scientific basis with which to approach the development of a consensus definition for IC. Examples of these efforts include: the work of the IC Epidemiology Task Force convened by NIDDK in October 2003; the work of the NIDDK Subcommittee on the Diagnosis of Interstitial Cystitis and Painful Bladder Syndrome, which presented its recommendations at the 2003 IC research symposium co-sponsored by the Interstitial Cystitis Association and is currently preparing them for publication; and the previously-mentioned planned initiative to validate APF as a diagnostic tool in larger studies.

Urinary Tract Infections: Urinary tract infections are a serious health problem affecting millions of people each year. Infections of the urinary tract are common; only respiratory infections occur more frequently. In 1997, urinary tract infections (UTIs) accounted for about 8.3 million visits to physicians. Most UTIs are caused by the common *Escherichia coli* (*E. coli*) bacteria. A UTI begins when bacteria enter the bladder, provoking an immune response and the sloughing off of bladder cells into the urine in the body's attempt to rid itself of offending bacteria.

Women are especially prone to UTIs for reasons that are poorly understood. One woman in five develops a UTI during her lifetime. Many women suffer from frequent UTIs: nearly 20 percent of women who have a UTI will have another, and 30 percent of those will have yet another. Recent research suggests that one factor behind recurrent UTIs may be the ability of bacteria to attach to cells lining the urinary tract. Bacteria can form a protective film on the inner lining of the bladder in mice, effectively "hiding out" from the immune system.

KIDNEY AND UROLOGIC DISORDERS OF CHILDHOOD

Chronic Kidney Disease in Children: Chronic kidney disease (CKD) is associated with numerous metabolic problems that can have significant negative impacts on the overall well-being of children with the disease. Growth impairment is one well-documented consequence of CKD in children, but there is less information about other developmental problems, such as impaired brain development and risk of cardiovascular disease. To address this lack of knowledge, in late 2002 the NIDDK, in collaboration with the National Institute of Neurological Disorders and Stroke (NINDS) and the National Institute of Child Health and Human Development (NICHD), solicited research applications for a prospective epidemiological study of children with CKD. In 2004, the National Heart, Lung, and Blood Institute (NHLBI) joined this initiative. Currently, the NIH supports two clinical centers and a data coordinating center that lead this study. The primary goals of this study are to determine the risk factors for the decline in kidney function in children with CKD; identify the risk factors for and incidence of impaired neurocognitive development and function; determine the prevalence of risk factors for cardiovascular disease; and examine the long-term effects of growth failure and its treatment in these children. The information obtained from this study will establish important natural history and outcome measures that will guide future intervention or prevention trials of pediatric CKD.

Research into Malformations of the Urinary Tract: During normal urination, the bladder contracts and expels urine through the urethra and out of the body. When this occurs, a valve-like structure at the back of the bladder closes to prevent urine from traveling back up the ureters toward the kidneys. However, in children with vesicoureteral reflux (VUR), this valve is either malformed or does not function properly, and some urine backs up into the ureters, possibly as far as the kidneys. This reflux can expose the kidneys to bacteria present in the urine, leading to kidney infection and possible kidney damage. Untreated reflux to both kidneys can, in the most severe instances, result in kidney failure, requiring dialysis or kidney transplantation. Because VUR is a congenital malformation, it is often first identified in children. Although some children will see their disease correct itself as they grow, others require surgery to address this condition. The NIDDK is currently acting on recommendations from a May 2003 meeting of the Vesicoureteral Reflux Task Force, which focused on the potential for conducting a randomized, controlled clinical trial in diagnosed children. The group identified several aspects of disease progression and treatment that are poorly understood and about which a clinical trial could provide important insights. In July 2004, the NIDDK issued a Request for Applications (RFA) for pediatric nephrology/urology clinical centers for the design and conduct of treatment trials and studies in affected children. The primary goals for this program are to study disease progression in a cohort of 600 children with mild to moderate disease and to determine which interventions are most beneficial. The NIDDK anticipates that planning for a clinical trial will begin in late 2005.

Other Studies of Congenital Urinary Tract Anomalies: The GU tract is the organ system most commonly affected by congenital birth defects, and many reports suggest that some of the birth defects may be increasing in incidence. Research into the normal development of the genitourinary tract is limited by a lack of cell-specific markers for key cell lineages within the developing GU tract, incomplete understanding of the normal three dimensional cellular structure of the major organs of the GU tract, incomplete understanding of the morphogenetic events that occur during organogenesis, and the lack of a detailed integrative database to assimilate complex temporal and spatial expression data. In late 2003, the NIDDK and NICHD solicited research applications for the development of a "Murine (mouse) Atlas of Genitourinary

Development." These projects would contribute to an anatomical and gene expression atlas of the developing mouse GU tract. When complete, the data from this atlas will greatly enhance scientists' understanding of events that occur during organogenesis (organ development). The atlas may lead to strategies for new and innovative approaches to corrective—and possibly preventive—strategies for these congenital abnormalities.

Congenital urinary tract obstruction, a condition termed uropathy, is one of the major causes of chronic kidney disease and ESRD in infants and children. The origins and best treatments of this disorder, however, are not well understood. To promote studies of these conditions, in March 2003, the NIDDK requested applications for basic and clinical research studies of obstructive uropathy. Among the goals of this effort are the development of objective prognostic markers; identification of genetic determinants of this congenital malformation; the development of reliable animal models of the malformation(s) to facilitate future research; and evaluation of the long-term effectiveness of various treatment strategies.

Pediatric Strategic Planning Task Force: As part of its planning process to guide future research areas of emphasis, in February 2005 the NIDDK sponsored a workshop that focused on assessing the "state of knowledge" and "future research needs" in areas related to pediatric urology. A multidisciplinary panel of clinical and basic science experts was convened to assess current scientific information related to pediatric urology and to develop suggestions for future research approaches. The topics for discussion in the workshop included many of the pediatric urological and nephrological developmental abnormalities, such as vesicoureteral reflux, ureteral abnormalities, and upper urinary tract obstruction.

It's Not the Shape, It's the Substance— NIDDK's Dr. Griffin Rodgers Offers Sickle Cell Update

For many years, doctors thought that the excruciating pain associated with sickle cell disease crisis was due to the sickled shape of the cell. The cells were contorted like sharp boomerangs rather than like friendly little disks and were thus tearing up the microcirculatory systems of patients.

In a recent talk at the NIH Clinical Center, NIDDK Deputy Director Dr. Griffin Rodgers described some of the key research advances and opportunities that can help to combat sickle cell disease, which in the U.S. occurs predominantly in people of African descent. For example, intramural research by investigators such as Drs. Connie Noguchi, Alan Schechter, William Eaton, and James Hofrichter at NIDDK helped to show that it is the chemical and biophysical properties of the hemoglobin within sickled cells, more than their shape, that impose the penalties of sickle cell disease. It is the accumulation of intracellular polymers, not the sickling of cells, that is principally responsible for pathogenesis. Dr. Rodgers holds out the hope of eventual stem cell or gene therapy as a cure for sickle cell disease, and illuminated gathering knowledge of the disorder, including recent studies by Schechter and his colleague Dr. Mark Gladwin, of the NIH Clinical Center, which implicate nitric oxide as a key contributor to the vascular constriction that is a hallmark of the disease.

"Sickle cell disease is one of the first diseases to be understood at the genetic level," said Rodgers. The disorder is caused by a single amino acid substitution and involves a reversible aggregation of sickle hemoglobin and eventual distortion of red blood cells, which can go back to their normal shape.

NIDDK Deputy Director Dr. Griffin P. Rodgers speaks at the NIH Clinical Center about NIH research into the causes of and treatments for sickle cell disease. Research supported by the NIH, and research conducted by NIH scientists at the Clinical Center, have contributed greatly to improvements in therapies for people with sickle cell disease.

There is a kind of parachuting effect that red cells undergo as they enter the microcirculation and erythrocytes loaded with polymer can't perform this function. Eventually, these cells can't traverse the microcirculation. Obstructions occur, affecting all organ systems. Rodgers called the disease's manifestations "protean" in that they involve neurologic complications, lung and liver ailments and periods of unrelenting bone and joint pain known as "musculoskeletal crisis."

Not every patient experiences the same level of severity; there are modifying factors owing to genetic and physiologic differences among patients. With respect to the former, scientists have been able to trace the migration of the sickle cell gene from the Old World to the New, more than 4,000 years ago. It appears to have originated in perhaps four sites in antiquity: Senegal (from which the least harmful condition emerged), Benin (home of an intermediate phenotype), India/Saudi Arabia (a mild, almost inconsequential version) and Bantu (associated with the most severe cases).

Several decades of NIH studies on fetal hemoglobin and its genetic control mechanisms have led to important discoveries. Dr. Rodgers and colleagues found that a cancer drug—hydroxyurea—can increase levels of fetal hemoglobin, thus moderating the disease's consequences. They launched the NIH Hydroxyurea Trial at the Clinical Center, in which patients remained at the hospital for up to three to four months while physicians tried escalating doses of the drug in a search for the optimal amount.

Most patients took two or three weeks to respond. While most experienced benefits, about 25-30 percent did not. Some began to feel better even before their fetal hemoglobin increased, so maybe there were other mechanisms at work.

In the 1990s, the National Heart, Lung, and Blood Institute funded a multicenter trial that was stopped early because, as a May 1995 article in the *New England Journal of Medicine* reported, there was a clear benefit to hydroxyurea therapy. It was associated with a 50 percent reduction in the frequency of painful sickle cell crisis, required less frequent blood transfusion, and reduced instances of "chest syndrome," a common cause of death in sickle cell patients. In 1998, the FDA approved hydroxyurea for use in sickle cell disease; it remains the only drug approved for that ailment. In 2003, the *Journal of the American Medical Association* published a nine-year follow-up study of patients taking hydroxy-urea. It showed that patients experienced continued increase in fetal hemoglobin levels, less acute chest syndrome occurrence and improved survival. The drug is now being actively studied in children with sickle cell disease.

Studies at the Clinical Center continue to refine the pathogenesis of hemolysis in sickle cell disease, and chemical factors affecting microvascular (or blood vessel) constriction. NIDDK's Schechter and Gladwin have shown how nitric oxide contributes to complications in the disease by regulating vasodilator tone and inhibiting platelet aggregation and adhesion, among other properties.

Dr. Rodgers envisions widening opportunities to intervene with drugs, and sketched the beginnings of an approach to a cure. Hematopoietic stem cell transplantation (HSCT) is one option researchers can pursue, but only one-quarter of patients have a suitable donor. Reduced intensity conditioning regimens followed by HSCT (non-myeloablative transplants) offer promise in adult patients in whom high doses of conventional preparative chemotherapy may prove unacceptably toxic. The Holy Grail is gene therapy, but unfortunately, that path is still a ways off. Improved methods are required to recognize true hematopoietic stem cells, to expand their number, and ultimately to have the replacement gene function in cells destined to become red cells. At the moment that's a difficult proposition. Cord blood might be useful as a source of hematopoietic stem cells for eventual gene therapy. Both the non-myeloablative and cord blood strategies are currently being pursued by Rodgers and his colleagues.

The NIH Clinical Center is an important venue for advances in sickle cell disease. The NIH will continue vigorous support of research toward a cure for this devastating disease.

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Sensing Calcium, Treating Disease

New treatments are emerging to correct abnormal calcium levels, which are common in the majority of patients who suffer life-threatening kidney disease and in people with certain rare diseases of the parathyroid glands, including parathyroid cancer. These treatments build upon substantial NIH investments in research that elucidated the role of the parathyroid glands in regulation of calcium levels. A new treatment strategy derives from the identification of the body's master regulator of blood calcium levels: the calcium-sensing receptor protein.

Because calcium is critical not only for bone formation but also for a myriad of other body functions, its levels are normally kept tightly controlled. In the 1960s and earlier, decades before the nature of the calcium-sensing receptor protein was defined, scientists studying large animal models found that low blood calcium levels cause the secretion of parathyroid hormone from parathyroid glands, while high blood calcium levels inhibit its release. Around 1960, NIDDK-supported scientists pioneered a method for preparing this hormone in a pure and stable form. They also developed what was then a new measuring technique, radioimmunoassay, to assay the very small quantities present in the human body. In the 1970s, these scientists devised a way to isolate cells from bovine parathyroid glands and grow them in the laboratory for study. These advances supplied the necessary tools for far more detailed experimentation.

From research supported by the NIDDK and others, scientists gained important insights into the regulation by calcium of parathyroid hormone secretion, the reciprocal influence of parathyroid hormone on calcium levels, and what happens when these processes go awry. Based on numerous studies over many years, scientists theorized that there is a calcium sensing mechanism on the surface of parathyroid cells that maintains constant surveillance of blood calcium levels. When calcium levels fall, this "calcium-sensing receptor" permits the secretion of parathyroid hormone, which then orchestrates a complex set of activities to help restore normal levels. These activities include the absorption of calcium from food in the intestines, its release from bones, and its reabsorption by the kidneys. When blood calcium levels become too high, the calcium-sensing receptor reins in parathyroid hormone. In diseases termed "hyperparathyroidism," this regulation is destroyed. Excess parathyroid hormone plunders the skeleton for its calcium, leaving bones more vulnerable to fracture and dumping potentially toxic amounts of calcium into the bloodstream. Patients may also experience fatigue, kidney stones, and impaired thinking.

In 1993, a group of scientists, funded in part by the NIDDK, identified the gene for the calcium-sensing receptor. Surprisingly, analysis of this gene revealed that the receptor is not, as previously thought, a channel through which calcium streams into cells. Rather, it is a novel member of a large family of proteins termed G protein-coupled cell-surface receptors. Because these proteins are prime drug targets for a number of health conditions, scientists had previously thought that the calcium-sensing receptor might be a good drug target for diseases marked by excess parathyroid hormone. Its landmark identification as a G protein-coupled receptor helped stimulate further research in this area.

With the gene for the calcium-sensing receptor in hand, scientists supported in part by the NIDDK discovered the underlying causes of some rare forms of "primary" hyperparathyroidism. People have two

copies of the calcium-sensing receptor gene. A mutation that reduces function of one copy causes reduced sensitivity to calcium in the parathyroids and kidney resulting in a mild, generally asymptomatic disorder termed familial hypocalciuric hypercalcemia (FHH). When mutations impair both gene copies, parathyroid cells are essentially totally unable to "sense" calcium, leading to a severe increase in secretion of parathyroid hormone. The resulting neonatal disease is severe, and removal of the parathyroids is required for babies to survive. This past year, researchers found that another form of hyperparathyroidism is an autoimmune disease: the body mistakenly produces antibodies that interfere with the calcium-sensing receptor's functioning. Other forms of primary hyperparathyroidism have been shown to result from excess—and sometimes cancerous—growth of parathyroid tissue.

Researchers have also, over many years, gained an understanding of "secondary" hyperparathyroidism, which is associated with kidney disease. When the kidneys fail, blood phosphate levels increase and calcium levels drop, as a result of loss of certain kidney functions important in calcium regulation, such as production of the active form of vitamin D, calcitriol. The body—in a doomed attempt to normalize calcium levels without healthy kidneys—then increases parathyroid hormone secretion. One result of secondary hyperparathyroidism is weakened bones, termed renal osteodystrophy in this context. Patients may also suffer from cardiovascular disease, likely related to disturbances in blood calcium and phosphate.

Treatments for hyperparathyroidism have not been ideal. Surgery to remove excess or abnormal parathyroid tissue has been, to date, the only effective way to treat primary hyperparathyroidism. Hyperparathyroidism related to kidney disease has been treated with phosphate binders, with calcium supplementation to increase its levels and thus suppress parathyroid hormone release, and by administering calcitriol, which also reduces the amount of parathyroid hormone. However, these therapies can result in high blood calcium levels and other problems. Surgery may then be necessary.

Research on the calcium-sensing receptor has now led to the development of a new drug by scientists at a biotechnology company. In 2004, investigators reported the results of an industry-sponsored clinical trial demonstrating the effectiveness of this oral drug in kidney disease patients on dialysis. It has been approved by the Food and Drug Administration for treating hyperparathyroidism associated with kidney disease. The drug is one of a novel class of compounds that interact with the calcium-sensing receptor in a way that "mimics" calcium. Called calcimimetics, they cause the receptor to perceive calcium levels in the blood as higher than they really are and thus reduce parathyroid hormone secretion. Calcimimetics, whose characterization was supported in part by the NIDDK, are also being explored for treating other forms of hyperparathyroidism, including high calcium levels resulting from parathyroid cancer.

Scientists are also currently investigating "calcilytics," compounds that have the opposite effect on the calcium-sensing receptor by leading to increased parathyroid hormone secretion. Paradoxically, parathyroid hormone can either weaken or help build bones—depending on the timing and extent of its secretion from the parathyroids. An orally-administered calcilytic may thus help treat osteoporosis by stimulating endogenous secretion of parathyroid hormone, obviating the need for injections of synthetic parathyroid hormone, recently approved by the FDA as a treatment to build bone.

NIH funding has contributed to a mosaic of vital advances, progressing from early basic research on parathyroid hormone and calcium regulation to the recent development of a new drug. Collectively, these discoveries represent a striking example of "translational" research, in which both NIH- and industry-supported investigators have benefited patients by propelling science from the bench to the bedside.

Jill Khederian

Fighting a Constant Battle Against Kidney Stones

Every time Jill Khederian leaves her home, she scans the horizon for the nearest restroom. "I have to drink so much water that every time I turn around I can pretty much tell you where any bathroom is," says the 53-year-old former post-anesthesia recovery room nurse and mother of three. Jill suffers from a painful and chronic condition called cystinuria, a rare, inherited disease that causes cystine stones to form over and over again in her kidneys. Cystine stones are difficult to treat and require life-long therapy. Drinking three to four liters of water a day helps prevent the creation of the stones, and when stones do form, helps to pass them through the body. But Jill's story is much more complicated than simply drinking lots of water to manage her disease. Left untreated, her condition could lead to end-stage kidney (renal) disease, requiring renal dialysis or a kidney transplant to survive.

Jill Khederian with her Jack Russell terrier, Sonya.

About Kidney Stones

Patients with kidney stones can experience one of the most painful of all disorders. Kidney stones affect the urinary tract. The urinary tract, or system, consists of the kidneys, ureters, bladder, and urethra. The kidneys are two bean-shaped organs located below the ribs toward the middle of the back. The kidneys remove extra water and wastes from the blood, converting them to urine. They also keep a stable balance of salts and other substances in the blood, and produce hormones that help build strong bones and help form red blood cells. Narrow tubes called ureters carry urine from the kidneys to the bladder, which expands to store the urine until it is emptied through the urethra to outside the body.

Kidney stones are hard masses that develop from crystals that separate from the urine as it is forming and build up on the inner surfaces of the kidney. Kidney stones are unrelated to gallstones, which form in a different area of the body. There are several different types of kidney stones, including calcium (the most common type), uric acid, struvite, and cystine. Urine contains chemicals that can normally prevent some types of crystals, or stones, from forming. These chemical inhibitors, however, do not seem to work for everyone, or they can be overwhelmed when there is an excess of a stone-forming agent in the urine. The good news is that most kidney stones pass out of the body without any intervention by a physician. However, the passing of a stone can be excruciatingly painful.

PATIENT PROFILE

Stones that are three millimeters or larger usually require more intensive treatments, including extracorporeal shock wave lithotripsy, or ESWL, the most frequently used therapy. In ESWL, shock-waves that are created outside the body travel through the skin and body tissues until they hit the stones, which are denser than the tissues. The stones break down into sand-like particles that are easily excreted in the urine. For stones that are quite large or in a location that does not permit effective use of ESWL, percutaneous nephrolithotomy may be recommended. In this procedure, the surgeon makes a tiny incision in the patient's back and creates a tunnel directly into the kidney to remove the stone. The least invasive treatments, however, are always the most preferred.

Kidney stones are not rare; they are one of the most common disorders of the urinary tract. An estimated 5 percent of adults in the United States have reported ever having a kidney stone, and men tend to be affected more frequently than women.

Many Americans may pass one kidney stone in their lives, but others may suffer from recurring, painful stones. Once a person gets more than one stone, others are likely to develop. The underlying cause for an individual kidney stone can vary from excess vitamin D in the diet to one of several metabolic disorders, and in some cases may remain unknown. While some causes of recurring stones are known, such as the inherited diseases cystinuria and hyperoxaluria (which causes calcium-oxalate stones), researchers are still teasing out what causes other patients to become highly susceptible to forming stones. A better understanding of the interplay between the diet, genetic predisposition, and metabolic dysfunctions that leads to the formation of kidney stones will also improve treatment and provide clues to prevention.

Living with Cystinuria

Cystine is an amino acid that does not dissolve well. Cystine is actually a special "di-amino acid" found in a number of proteins, in which it acts as a stabilizing unit. Small amounts of cystine and other amino acids enter the blood from the diet and from protein metabolism. When blood is filtered through the kidneys to remove wastes, cystine and the other amino acids get filtered, too. Normally, the kidneys reabsorb amino acids and other non-waste molecules while urine is being formed. In Jill and other people with cystinuria, the disease gene causes high levels of cystine in the urine. The excess cystine forms crystals that develop into cystine stones. The danger of not being able to pass these stones is that they can block the flow of urine, causing ongoing urinary tract infection and damage to kidney tissues, as well as cause constant bleeding. Trying to prevent cystine stones from developing is important because medical management is less successful with cystine stones than with more common types of kidney stones. However, prevention is more easily said than done.

In 1970, Jill was a 19-year-old college sophomore when she passed her first stone. "I was living off campus with a friend when I began feeling this pain," says Jill. The pain began as an ache in her back and side. Then, it became more constant and severe as her urinary system tried to rid itself of the stone. She experienced a burning sensation during urination and blood in her urine, as well as a frequent urge to urinate. She became nauseated and began vomiting, and her lower abdomen was painful when touched. Jill, who has passed several stones since, describes the pain as "like you've been hit by a car," a gripping pain that even painkillers can barely ameliorate. "I've given birth to three children, all [without drugs]" she says, "and that doesn't even compare to passing a kidney stone."

The most common symptoms of kidney stones include:

- **Extreme, sharp pain in the back or side that will not go away**
- **Blood in the urine**
- **Nausea and vomiting**
- **Cloudy or odorous urine**
- **Frequent urination**
- **A burning feeling when you urinate**
- **Fever and chills**

The stone Jill passed at age 19 was found to be 100 percent cystine. Subsequently, she was diagnosed with cystinuria. Neither of her parents suffers from the disease; her father has passed a couple of kidney stones, but they were calcium stones. Yet, she has learned that both parents are carriers of the gene that causes cystinuria. Jill's older brother, John, also has the disease. Because both biological parents need to carry the gene to pass the disease on to their children, Jill's three daughters most likely will be spared from developing cystine stones because their father, Jill's husband, is not a carrier.

Treating Cystinuria

Jill underwent her first major surgery in 1977 when a stone obstructed one of her ureters, which are the tubes that conduct urine from the kidneys to the bladder. As a result of the blockage, her left kidney began to swell. "I was told I needed a left pyelolithotomy, a surgical procedure to remove the stone, or I might lose a kidney and possibly die!" says Jill. Even major surgery failed to remove the entire stone. "Because of its location and hardness, physicians were able to remove most, but not all of it," adds Jill.

Despite their small size, recurring kidney stones can pose tremendous difficulties for patients, as Jill has found. Over the years, she has taken several different medications to try to prevent cystine stones from developing in her kidneys. Some have resulted in side effects, including skin rashes and swollen joints in her fingers. She is currently on 1,200 milligrams a day of tiopronin. This drug changes the chemical composition of cystine, and is prescribed to prevent cystine stones from forming. Unfortunately, the drug has a relatively short shelf life, is expensive, and—like many medications—has potentially severe side effects that need to be monitored by a physician. Jill has also undergone serious medical procedures for her condition over the past five years, including three laser surgeries, one of which resulted in a three-day hospital stay.

Research on Kidney Stones

While there are multiple causes and types of kidney stones, the end result is the same: an imbalance in urine components that leads to a painful precipitate that can damage the kidneys and harm urologic function. Researchers are working on finding and developing new drugs with fewer side effects to prevent or treat kidney stones. In addition, the growing field of lithotripsy has greatly improved the treatment of kidney stones. However, researchers continue to seek answers to questions such as:

- Why do some people continue to have painful stones?
- How can doctors predict, or screen, those at risk for getting stones?
- What are the long-term effects of lithotripsy?
- What role do genes play in stone formation?
- What other natural substance(s) found in urine can block stone formation?

PATIENT PROFILE

Cystinuria is a lifelong condition causing recurrent kidney stones. The rate of stone formation can sometimes ease up as patients get older. Unfortunately, Jill has not experienced symptom reduction, which makes prevention even more important for her. Morever, she is very concerned that she might follow in her brother John's footsteps. He underwent an operation in the 1980s that uncovered hundreds of cystine stones in his kidneys. Like a number of kidney stone sufferers, he has experienced serious kidney complications. "My brother is four years older than I am. He has high cholesterol, high blood pressure, permanent damage to his left kidney, and he just recently developed anemia, all of which is believed to be related to his cystinuria. He's been told that within a year it's very likely he will need dialysis," Jill says.

Fortunately for Jill, right now her blood pressure is low to normal; her cholesterol is fine; and she shows no signs of kidney failure. To stay in shape, Jill exercises regularly. And to do what she can to prevent the recurrence of her cystine stones, she flushes her system with plenty of water. "I'm always aware of my need to be drinking water," she says.

Many of the advances in kidney stone treatments, including those for cystinuria, have resulted directly from NIDDK investments in research to understand the underlying causes of kidney stone disease. Translation of basic understanding of kidney stone disorders into applicable treatments or cures is the ultimate goal for research in this area. The NIDDK continues to strengthen its research program on the causes and potential treatments for recurring kidney stones. For example, recurring calcium oxalate stones, the most common form of kidney stones, are caused by inherited metabolic diseases such as the primary hyperoxalurias, but more commonly occur "spontaneously"—though with some familial tendency. It is suspected in the latter case that there are unique genetic, environmental, and/or metabolic factors that predispose individuals to recurrent calcium oxalate stone formation. A current NIDDK initiative is encouraging innovative research on all forms of calcium oxalate stone disease in an effort to shed light on pertinent metabolic, molecular, and genetic defects, and to eventually develop more effective diagnostic, treatment, and preventive strategies. Studies to determine the contribution of genetic factors to kidney stone formation in the general population will also be helped by information and research tools evolving from the NIH investment in the Human Genome Project. The Institute is also pursuing a research agenda in hereditary calcium oxalate stone disease, in order to address the special needs of this patient group.

The National Kidney Disease Education Program (NKDEP)

An estimated 10 to 20 million Americans currently suffer from kidney damage, also called chronic kidney disease. Each year, over 300,000 must have kidney dialysis to stay alive. The number of people developing irreversible kidney failure, also called end-stage renal disease (ESRD), has doubled each decade for the last two decades, and disease statistics indicate that this trend is likely to continue. The leading causes of kidney disease are diabetes and high blood pressure. If current trends continue, the recent increases in obesity and type 2 diabetes in the U.S. will have grave implications, as more and more people develop kidney complications related to diabetes. The public and private costs of treating ESRD were estimated at $23 billion in 2001.

Fortunately, chronic kidney disease can be slowed, if not prevented, provided it is detected early. Good control of blood sugar and blood pressure can reduce the risk of developing kidney disease. Diets low in protein can also slow kidney disease progression. In spite of these advances in treatment and prevention, only a small number of people who most need proper screening or treatment receive it. The NKDEP strives to disseminate information on prevention and treatment to physicians and patients who can most benefit from it.

Racial and ethnic minorities suffer a far greater incidence and prevalence of irreversible kidney failure than Caucasians. Rates of ESRD are disproportionately greater in African Americans, American Indians and Alaska Natives, Native Hawaiians and other Pacific Islanders, and Hispanic Americans. Diabetic kidney disease is the most common cause of ESRD in all of the minority groups except for African Americans, in whom high blood pressure-induced kidney damage is also a major cause.

The ultimate goal of the National Kidney Disease Education Program (NKDEP) is to reduce complications and death due to kidney disease and kidney failure among all Americans. Currently, the NKDEP is targeting primary care providers and people at high risk for kidney disease—particularly African Americans with diabetes, high blood pressure, or a family history of kidney failure. In June 2004, the NKDEP nationally launched the campaign, "You Have the Power to Prevent Kidney Disease," which emphasizes three key messages:

- Early detection is important. If you are at risk due to diabetes, hypertension or a family history of kidney failure, talk to your doctor about having your kidneys checked.
- Effective treatment can prevent or slow kidney damage.
- Left undiagnosed and untreated, kidney disease can lead to kidney failure.

The program plans to broaden its target audiences to include other at-risk audiences as the program expands. Prior to launching the national campaign, NKDEP piloted the program in four cities: Atlanta, GA; Baltimore, MD; Cleveland, OH; and Jackson, MS. Successful strategies identified through the pilot sites were used to inform the national campaign.

In addition to public awareness activities, the NKDEP has several Work Groups that are striving to remove specific barriers to better kidney disease awareness and care. The membership of these groups is drawn from the professional partnership network of the NKDEP, which includes non-profit groups, industry, and government. The NKDEP Laboratory Work Group has made efforts

to encourage improvement and standardization of the serum creatinine assay–which is used to estimate how well the kidneys are functioning—in order to address issues of inter-laboratory variation in this assay. The group has also begun efforts to encourage laboratories to report glomerular filtration rate (GFR) estimates as soon as possible in adults with low GFRs, to enable physicians to quickly identify individuals with impaired kidney function. The NKDEP Quality Indicators Working Group, in partnership with the Centers for Medicare and Medicaid Services (CMS), is undertaking a pilot project to spur the development of quality indicators of care for chronic kidney disease among Medicare beneficiaries hospitalized for cardiovascular disease.

Through all of these efforts, the NKDEP is a positive force in helping to reduce the burden of kidney disease in the U.S.

Molecular Basis of Urinary Tract Infections: More to the Picture than Meets the Eye

Dr. Scott J. Hultgren

The NIDDK National Advisory Council meets three times annually to provide advice to the Institute regarding its research portfolio and broad issues of science policy. These meetings are also an opportunity for the Council members to learn about recent scientific advances in different fields through presentations from NIDDK-supported extramural scientists. In September 2004, the Council and NIDDK staff were privileged to hear from Dr. Scott J. Hultgren, a leading researcher in the field of bacterial pathogenesis. Dr. Hultgren is the Helen L. Stoever Professor of Molecular Microbiology at the Washington University School of Medicine in St. Louis, Missouri. He received his Ph.D. at Northwestern University in Chicago in 1987, and conducted his postdoctoral studies at Umeå University in Sweden until 1989. Dr. Hultgren's research team is pushing to decipher the molecular crosstalk that takes place between pathogenic bacteria and cells in the bladder and to translate this information into possible therapies for recurrent urinary tract infections. The following highlights are adapted from his presentation to the Council.

Like marauders at the castle gates, microbes seeking to colonize a host must battle and prevail against host defenses before they can reach a safe haven. Stealth, timing, and complex biological activities at the cellular level all come into play. In the case of bacteria that colonize the bladder and cause urinary tract infections, exciting results are emerging from in-depth study of these activities at the host-pathogen interface. Not only do these bacteria activate myriad innate host defense systems, but recent studies also suggest that one result of infection is bladder cell turnover and proliferation—an observation that may, in turn, provide insight into pathways important in bladder cancer. Moreover, molecular pathways are activated in the bacterium upon its interaction with the host. These activated pathways enable the bacterium to subvert innate host defenses, persist in the urinary tract, and cause disease. UTIs are quite prevalent: an estimated 34 percent of adults, mostly women, have had at least one urinary tract infection. Understanding both bacterial tricks and host defenses in bladder infections may ultimately result in new and more effective treatments for these infections, prevent their recurrence, and uncover important aspects of bladder biology.

A Sticky Pike: The Pilus

Escherichia coli, or *E. coli*, is a rod-shaped bacterium normally found in the colon, where it aids the body in the last stages of digestion. However, some *E. coli* acquire features that significantly enhance their ability to survive and cause disease if they are accidentally introduced into the urinary tract. These strains of bacteria are called uropathogenic *E. coli*, or "UPEC."

Most bacteria that reach the bladder will get flushed out with the urine. UPEC, however, use hair-like fibers with sticky tips, called pili, to adhere firmly to cells lining the bladder. While pathogenic *E. coli* may have more than one type of pilus on their surfaces, the pilus important for UPEC attachment to bladder

cells is called the "type 1" pilus. From studies spanning a number of years, a detailed molecular picture has emerged of how the type 1 pilus and similar adhesive pili are constructed. This knowledge has provided insight into the strength of these structures so important to UPEC pathogenesis, and into ways to disrupt their assembly.

In a series of steps called the "chaperone-usher" pathway, bacteria assemble the type 1 pilus and similar pili from an array of pilus subunit proteins. The role of the "chaperone" protein in this bacterial pathway is to grab the immature pilus subunits and prepare them for assembly into a complete pilus. To carry out its role, the chaperone employs an unusual molecular tactic: after making contact with a pilus subunit, the chaperone guides the subunit to assume its proper three-dimensional structure by temporarily providing it with a missing piece that completes the structure. Chaperone-subunit complexes then move to a pilus assembly site at the bacterium's outer membrane, known as an "usher." As the pilus subunits are assembled, they interlock like Lego™ pieces—each subunit now providing to the next the missing structural element originally supplied by the chaperone. The nascent pilus is channeled through the usher to the surface of the microbe, where it can assume its final, functional conformation, which is very stable and strong.

A key finding with potential implications for anti-microbial, including anti-UPEC, therapy has emerged from this elucidation of pilus assembly. The chaperone-usher pathway is shared by *E. coli* and many other species of mostly disease-causing bacteria, ranging from those that cause plague to those that cause urinary tract infections. In the hundreds of bacterial species that have been studied, the chaperone proteins all possess a conserved molecular site in the part of their structure that first contacts the pilus subunits. This site is crucial to the chaperone's ability to interact with the subunits. If this site is altered, the pilus subunits cannot be assembled and they degrade. The importance of this site to chaperone activity and pilus assembly, as well as its conservation among so many species, makes it an attractive target for therapy. Currently, Dr. Hultgren's research team is working to design a drug that would bind to this site in the chaperones and inhibit the assembly of adhesive pili—thereby severely diminishing the capacity of piliated bacteria to cling to and colonize host tissues, such as the bladder. Such a drug could have broad spectrum activity against a wide range of pathogenic bacteria.

Bladder as Battlefield

When UPEC reach the bladder, they meet a complex organ. The bladder is composed of several layers of epithelial cells, connective tissue, and muscle. The cell layers most involved in interactions with UPEC, however, are the superficial umbrella cells—which constitute the top inner layer of the bladder—and the intermediate, or transitional, epithelial cells, which rest on the basal cells. The superficial umbrella cells are terminally differentiated, meaning that they have attained a specialized functional state and can no longer proliferate. However, the underlying transitional cells are less mature and are responsible for renewing these specialized cells as needed.

The superficial umbrella cells are perhaps some of the largest cells in the body, and they produce very interesting proteins called uroplakins. Studies of these proteins by another NIDDK-supported research team have shown that there are four uroplakins, which assemble in groups of six into hexagonal structures with a central "pore." The hexagonal particles are then further organized to form a crystalline plaque-like material with a dense honeycomb appearance, which coats the surface of the bladder. This uroplakin coating forms an impermeable surface to prevent the leakage of toxic molecules from the urine into the bladder epithelium.

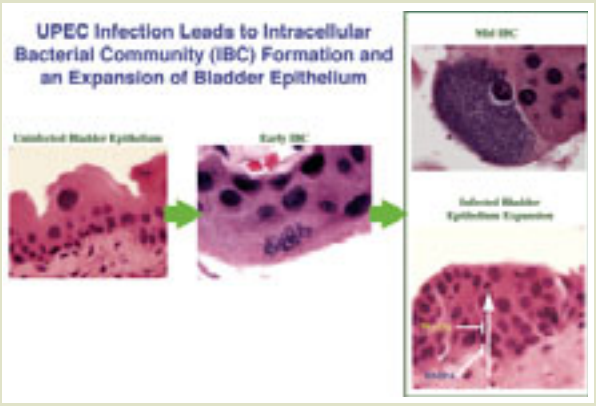
Yet, while uroplakins are meant to be a shield, in the case of attack by UPEC, they are also an Achilles heel. UPEC elaborate type 1 pili that are able to

bind to a specific sugar—mannose—that is present on the uroplakins. A protein present at the tip of the type 1 pilus, called FimH, is key to recognizing the mannose molecule. Using high resolution electron microscopy, Dr. Hultgren's research team has observed the tips of the type 1 pili fibers buried within the mannose-rich central pore of the uroplakin particles. This finding confirms visually other experimental data that suggested that interaction between the type 1 pilus adhesive tip and the uroplakin proteins is the initial step in UPEC attachment to bladder cells.

UPEC attachment is met with firm resistance, however. One of the most dramatic consequences of this host-pathogen interaction is that it activates a pre-programmed, self-destruct sequence in the superficial umbrella cells. Activation of this mechanism leads the affected cells to slough off the inside of the bladder wall in about 12 hours. Other changes occur, too. Normally, the bladder is very quiescent, and regeneration of bladder tissue is very slow, occurring only maybe every six months to a year or so. But upon UPEC infection, normally silent regeneration pathways are activated within hours, allowing the underlying transitional cells to proliferate and differentiate to replace the exfoliated superficial umbrella cells. This process likely represents a very potent innate defense; by exfoliating and replacing the exposed superficial umbrella cells, the bladder can rid itself of contaminated cells with minimal tissue injury. Finally, assault by UPEC triggers the bladder cells to signal for assistance from immune system cells, called neutrophils, which flood into the bladder to fight the bacteria. The bacteria thus need to subvert these defenses in order to survive in the urinary tract.

Differentiation in Host Defense

Building upon the knowledge about the interaction between the FimH protein and uroplakins, Dr. Hultgren's research team was able to probe beneath the surface to find out what happens within the bladder cells that drives their renewal in reaction to UPEC colonization. One experiment looked for genes that were turned



Consequences of bladder infection by pathogenic *E. coli* bacteria. Left panel, uninfected bladder cells stained pink with purple nuclei; center panel, bladder cells (light purple with dark purple nuclei) with an early infection of tiny dark blue bacteria; right panel, bottom, proliferating bladder cells stained light pink with dark purple nuclei; right panel, top, a bacterial network visible within a single bladder cell as tiny dark blue specks to the left of the cell's nucleus.

"on" or "off" in mouse bladder cells when mice were infected with two different laboratory strains of UPEC. One strain had a normal FimH, and the other lacked FimH and could no longer adhere to mannose. This experiment revealed about 50 genes that are differentially regulated in the bladder cells infected with the normal UPEC. Many of the genes identified are involved in pathways governing cellular differentiation and proliferation. Two of the cellular pathways that came to light from this study are the "BMP4 pathway" and the "sonic hedgehog" pathway.

The BMP4 pathway is a very complicated cellular pathway that, when activated, leads to signaling cascades that ultimately inhibit cellular differentiation in many developmental systems. Results from two different types of experiments indicate that UPEC infection induces down-regulation of this pathway within a few hours, whereas infection with the FimH mutant UPEC has no effect. These results suggest that the BMP4 pathway normally acts to suppress unnecessary differentiation of transitional cells, but is deactivated upon UPEC infection in order to allow

the regeneration processes it governs to be activated. Similarly, a few hours after infection, sonic hedgehog—an activator of development—is up-regulated in bladder cells, potentially promoting regeneration. These tantalizing results have launched a new set of studies to capture the molecular crosstalk at the host-pathogen interface and determine the precise molecular mechanism(s) by which UPEC infection activates proliferative pathways and represses inhibitors of proliferation.

These findings may also shed light on growth pathways involved in bladder cancer. Infections with some species of bacteria, such as the *Helicobacter pylori* bacteria that are associated with stomach ulcers, have been implicated as predisposing factors for certain cancers. Analysis of patients with bladder carcinoma has revealed that 60 percent of their tumors have deletions in a specific region of chromosome 9. This finding suggests that genes encoding so-called "tumor suppressors" exist in this region of the chromosome. Interestingly, a gene encoding one of the components of the sonic hedgehog pathway is found within the same region of chromosome 9. Thus, study of cellular proliferation pathways activated in response to UPEC infection in animal models may also uncover heretofore unrecognized connections between bacterial infection and bladder diseases, such as bladder cancer, that are associated with dysfunctional regulation of cellular growth and differentiation—or, simply provide a tool to identify the relevant pathways. Studies are under way to investigate in mouse models whether deleting the genes identified in the normal and FimH mutant UPEC-infected mouse screen will lead to bladder tumor formation.

Invasion and Evasion by *E. coli*

The host pathways that are activated when UPEC interact with bladder tissue represent potent innate defenses. So the question remains, why is urinary tract infection such a problem in the clinic? It appears that the bacteria have evolved mechanisms to subvert these innate defenses so that they can persist and cause disease.

While the interaction between type 1 pili and a bladder cell triggers host defenses, another consequence of attachment is that it occasionally triggers the "zippering" of the superficial umbrella cell around the attached UPEC bacterium—a desirable event from the bacterium's perspective. Once inside the cell, the successful bacterium starts a race against time to ensure its survival and eventual spread to new host cells. UPEC can actually invade both superficial umbrella cells and the underlying transitional cells; however, they undergo an unusual growth pattern in the former, and an intriguing series of events begins to unfold. These events have been captured in "real time" using time-lapse video microscopy to film UPEC-infected cells in mouse bladders over the course of infection.

After entering superficial umbrella cells, UPEC proliferate rapidly, transitioning from a single rod-shaped bacterium to a developmental stage called an early "intracellular bacterial community" (IBC). These early IBCs are loose collections or "clumps" of typically rod-shaped *E. coli*. After about 6 hours, however, the UPEC in these early communities change dramatically, transitioning to a mature "mid-IBC" stage. In this stage, bacterial growth slows, the bacteria become rounded, and the chaotic clump becomes a dense, organized population embedded in a matrix of polysaccharides (chains of sugar molecules). This fascinating development and its similarity to a unique type of bacterial growth led the mid-IBCs to be dubbed "biofilm-like" networks. In mice, the mid-IBCs—filled with up to one million UPEC—actually press the surfaces of their host cells outward and can be seen as uroplakin coated "pods" on the inner surface of the bladder. Finally, a few hours after the mid-IBCs form, the bacteria undergo another change, in which they start to detach from the network, become motile, and "flux" out of the host cell to reinitiate the invasion cycle in other bladder cells.

The organized bacterial networks formed by the mid-IBCs are especially interesting—and enlightening—due to their similarity to biofilms. Biofilms are special bacterial communities often associated with bacterial

persistence in a variety of environments, including diseased human tissues and medical devices. They are particularly resistant to antibiotics and to host immune responses. This resistance is thought to be facilitated by two key features of biofilms: the formation of the protective polysaccharide matrix and the development of "community behavior." Through the latter, the bacteria work together to sense and react to the environment, both to protect themselves and to enable spread into new environments. As a biofilm matures, the bacteria often form distinguishable subpopulations that are thought to serve distinct functional purposes in this community, similar to bees in a hive. Variation in the population also enhances the likelihood that at least part of the population could survive changes in the environment.

The observed generation of intracellular biofilm-like communities during UPEC infection has raised a number of questions, such as, what are the critical molecular signals controlling the maturation event? And, what signals tell the bacteria to stay put in the IBC matrix, and then disperse at the right time and in a directed, orderly fashion? There are a number of hypotheses about host signals that are involved, as well as signals elaborated by the bacteria that may enable them to work and communicate with each other in the IBC. For example, Dr. Hultgren's research team hypothesized that genes involved in pathogenesis—such as those important for making the type 1 pilus—are likely to be involved in the IBC activities. The team conducted experiments to analyze gene expression from the genetic element that controls the genes for the type 1 pilus subunits. The results of these experiments suggest that expression of the type 1 pilus genes increases over time in bacteria within the IBCs. This view was confirmed by using high resolution microscopy to peek inside the pods, which revealed that every bacterium is entirely coated with fibers, many of which appear to be type 1 pili. Interestingly, the fibers are not so much interacting with other bacteria, as much as they are interacting with the surrounding matrix. This interaction may potentially help to organize the bacterial network in its very defined biofilm-like array—and thus, possibly fulfill multiple roles in UPEC pathogenesis.

Ultimately, to understand the behavior of the biofilm-like IBCs, it will be important to be able to capture and understand the gene expression profile for each bacterium in the biofilm. Dr. Hultgren's research team is developing techniques to determine, for example, which other bacterial genes are also "on" or "off" during type 1 pilus gene expression. Another interesting aspect of these studies will be to discover the role of these IBCs in generating diversity in fitness—that is, how well they prepare the UPEC so that, as the bacteria emerge from the network and host cell, they are more fit to deal with stresses and to spread in the urinary tract and in the environment.

One interesting adaptation by UPEC to the hostile environment of the inflamed bladder is filamentation. When the late-IBC UPEC begin to flux from the host cell, many return to their normal rod shape. Some, however, develop into tremendously long rods—sometimes over 20 times their normal length. These filamentous bacteria are apparently impervious to attack by the immune system neutrophils, but still competent to invade new host cells. Already stymied by the protective, uroplakin-coated IBC pods, the neutrophils are thus further handicapped in their ability to clear the UPEC infection. Thus, the IBC pathway is both a protective and an adaptive response used by the bacteria in their quest for survival in the bladder.

Lurking Bacteria

After second and third generations of IBC formation, something interesting happens. The intracellular replication stops. The bacteria, after about two weeks, stop dividing altogether. They no longer replicate and no longer form biofilm-like intracellular communities. However, bacteria can co-exist in a cell and persist for months in this quiescent state.

Observation of this curious phenomenon has led to a key hypothesis: that the quiescent bacteria comprise a reservoir that may explain recurrent urinary tract infections in humans. In humans, a key diagnostic tool for UTIs is the presence of cultivatable bacteria

in the urine. Absence of bacteria in the urine following antibiotic treatment is thought to indicate successful clearance of infection. However, the urine of mice that are harboring quiescent UPEC bacteria is also sterile—despite the fact that their bladders are infected. If what is true of the mouse model is also true in humans, a lack of bacteria in the urine might not necessarily mean a lack of bacteria in the bladder. Thus, the urine culture may actually be missing bacteria lurking in the bladder epithelial cells, awaiting a signal to emerge and reinitiate an active infection.

If UPEC can exist in a quiescent state in human bladders and through this route are indeed responsible for some portion of recurrent UTIs, one possible trigger for their reactivation could be the regeneration of the cells lining the bladder. This regeneration requires the underlying cells to start proliferating. Experiments have revealed that the UPEC not only like to form IBCs in superficial umbrella cells, but that they will also form them in underlying cells that have been artificially stimulated to proliferate—cells that normally do not support IBC formation. Thus, these observations have laid the groundwork for other studies seeking to determine the clinical relevance of intracellular UPEC.

Translating from the Laboratory Bench to the Bedside and Back Again

The current clinical paradigm for recurring UTIs is that *E. coli* is an extracellular pathogen in the urinary tract and that recurrence of infection is always due to a "fresh" inoculation from an external source, such as the colon. The preceding findings suggest a very different paradigm for urinary tract infections, in which invasion of bladder cells by UPEC is critical to the ability of these bacteria to persist in the bladder, cause disease, and quite possibly to cause recurrent infections. Dr. Hultgren, his research team, and collaborators are pursuing the clinical relevance of these findings. In a current study, women who have had at least one recurrence of a UTI are being monitored regularly for bacterial load in the urine and for whether they have a recurrence. UPEC isolated from these women are being inoculated into mouse strains to determine whether they form IBCs. At the same time, the clinically isolated bacteria are being examined to pinpoint genetic differences among them that are associated with differences in their ability to form IBCs or with variations in UTI symptoms. Thus, applying knowledge gained from a fundamental understanding of how UPEC interact with an animal host to the analysis of actual clinical infections may translate into both a better understanding of UPEC pathogenesis and improved treatments for UTIs.

Hypotheses to Pursue

So far, much has been discovered about the structural basis of the initial host-pathogen interaction, how that leads to the activation of the IBC developmental pathway, and how these intracellular communities are the home for generating diversity in the UPEC bacterial populations so that when they flux out of the host cells, they are possibly more fit to colonize the urinary tract as well as the environment. The relevance of the UPEC reservoir in bladder cells to recurrent UTIs remains a key clinical question. Clues as to the signal(s) for the reactivation of these reservoirs back into acute infections with rapid growth and IBC formation are beginning to emerge. Finally, understanding the overlap between infection and regeneration processes, and how that may be important in not only normal bladder development, but abnormal bladder development, such as bladder cancer, is an important avenue to pursue.

In conclusion, the work in the Hultgren lab seeks to understand the molecular details of each step of an encounter between a pathogenic bacterium and its host tissue. Dr. Hultgren's research program integrates multiple disciplines, ranging from innovative translational research to providing snapshots of molecules caught in the act of triggering disease processes. His work is leading to a better understanding of UPEC infection, sparking better therapies to treat chronic and recurrent infections, and generating models valuable for the study of an array of human diseases.

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Top row (bench)—third photo from left (of two scientists facing right toward lab bench)—Richard Nowitz, for NIDDK; others photos: Getty Images
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